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(54) Title: MAMMALIAN RECEPTORS FOR INTERLEUKIN-10 (IL-10)

(57) Abstract

Mammalian IL-10 receptor subunits are provided, together with nucleic acids encoding various species variants of the subunits. Uses of the nucleic acids and receptor subunits are also provided, including methods for screening for agonists and antagonists of the receptor ligands, methods for producing diagnostic or therapeutic reagents, and methods for producing antibodies. Therapeutic or diagnostic reagents and kits are also provided.

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MAMMALIAN RECEPTORS FOR INTERLEUKIN-10 (IL-10)

The present invention relates generally to nucleic acids and polypeptides characteristic of receptors for mammalian interleukin-10, and more particularly to their use in preparing reagents useful for diagnosing or treating various receptor-related medical conditions.

BACKGROUND OF THE INVENTION

Activated hemopoietic cells secrete numerous proteins, some of which are called cytokines. Cytokines play a variety of important roles in regulation of immune responses by controlling proliferation, differentiation, and the effector functions of immune cells.

The actions of cytokines are typically mediated by specific receptor molecules found on target cells. The structure and mechanism of action of these receptors on target cells are not well understood, although it is known that many are composed of at least two separate polypeptide chains.

One chain, typically designated the α chain, can bind its cytokine ligand with low affinity. This interaction may or may not result in transduction to the cell of a signal. Another chain, designated the β chain, when associated with the α chain, confers higher affinity binding of the heterodimeric receptor to the cytokine. The β chain by itself usually lacks significant ligand binding affinity. The dimeric form of receptor is capable of transducing a signal into the cell as a consequence of ligand, e.g., cytokine, binding.

One cytokine, which inhibits the synthesis of a number of other cytokines, is called interleukin-10 (IL-10). See Fiorentino *et al., J. Exptl. Med. 170*:2081 (1989); and Mosmann et al., *Immunol. Today 12*:A49-A53 (1991). Both mouse and human counterparts have been isolated. See Moore, *et al., Science 248*:1230 (1990); and Vieira, *et al., Proc. Nat'l Acad. Sci.* USA *88*:1172 (1991).

A human viral analog, known as either vIL-10 or BCRF1, has been described which shares many characteristic activities of the natural human form. See Hsu *et al., Science 250*:830 (1990). Another viral homolog has been described from an equine herpes virus. See Rode *et al., Viral Genes 7*:111 (1993).

Because of the biological importance of IL-10 and because IL-10 acts by first binding to celular receptors, there is need for isolated components of such receptors, and for materials and methods for making and using such components.

SUMMARY OF THE INVENTION

The present invention fills these needs by providing nucleic acids and protein sequences of components of a receptor for IL-10. Both a human IL-10 receptor component and a mouse counterpart are exemplified, though equivalent components from other mammalian species will be found by similar methods or based upon other properties derived therefrom.

More particularly, the present invention provides recombinant or isolated nucleic acids comprising a sequence exhibiting homology to a sequence encoding a mammalian receptor for IL-10, a fragment thereof, or a unique portion thereof. In preferred embodiments, the nucleic acids will comprise deoxyribonucleic acid, will be isolated, further comprise a regulatory sequence from the 5' or 3' sequence adjacent a gene encoding a receptor for IL-10, or are operably linked to a genetic control element. In alternative embodiments the receptors, fragments, or portions thereof have a biological activity, e.g., one characteristic of a receptor for IL-10, or are from a mammal, including a mouse or human.

In particular embodiments, the nucleic acids, are capable of hybridizing at high stringency to SEQ ID NO: 1 or 3, or are isolated using a probe which hybridizes at high stringency to a human receptor gene for IL-10. The invention also embraces nucleic acids capable of hybridizing to these sequences, e.g., which contain mutations selected from the group consisting of nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. Alternative embodiments include

recombinant nucleic acids which are operably linked to a genetic control element, e.g., a prokaryotic promoter element or a eukaryotic expression control element, including a viral promoter.

Various embodiments include expression vectors for expressing DNA encoding a receptor for IL-10, or fragments thereof, or vectors comprising these sequences and a selection marker. The invention also embraces host cells comprising an expression vector which is capable of expressing these receptors. Preferred host cell embodiments include prokaryotes, including gram negative and gram positive bacteria, including *E. coli*; lower eukaryotes, including yeasts; and higher eukaryotes, including animal cells such as mammalian and primate cells, including human. Preferably the receptor is selected from a human receptor for IL-10; or a mouse receptor for IL-10. Other embodiments include nucleic acids further encoding a second protein or polypeptide, e.g., where the second polypeptide is fused to the receptor or a fragment thereof. The invention further embraces subcellular structures, cells, or organisms comprising these nucleic acids.

The present invention also embraces proteins or polypeptides encoded by these DNA sequences, preferably which are substantially free of protein or cellular contaminants, other than those derived from a recombinant host. The receptor proteins or polypeptides will often be from a mammal, including a mouse or human, and can have an amino acid sequence as found in SEQ ID NO: 2 or 4, or an allelic or species variant thereof, or a unique portion thereof. The receptor proteins or polypeptides can be attached to a solid support, be substantially pure, or be in a pharmaceutically acceptable form, with or without additional carriers or excipients. The invention also conceives of fusion proteins or polypeptides, including those further comprising a sequence from a second receptor protein. Other embodiments include subcellular structures, cells, or organisms comprising such receptor proteins or polypeptides.

The invention also provides methods for producing receptor proteins or polypeptides comprising culturing a cell comprising a described nucleic acid in a nutrient medium; and expressing the receptor proteins or polypeptides in the cell. Various alternative

embodiments further comprise a step of purifying the receptor proteins or polypeptides, where the receptor proteins or polypeptides are secreted into the medium and purified therefrom, and wherein the receptor is from a mammal, including a mouse or human. The invention also provides receptors made by these methods and exhibiting a post-translational modification pattern distinct from that in normal native receptor, e.g., glycosylation; alkylation; and carboxylation. The receptor can be made in a cell line expressing a receptor exhibiting a non-natural receptor glycosylation pattern. The invention also provides methods for diagnosing a medical condition characterized by inappropriate IL-10 response in a host comprising contacting a sample from the host with a specific binding reagent to (i) a nucleic acid encoding a receptor for IL-10 or fragment thereof; or to (ii) a receptor for IL-10 or fragment thereof, and measuring the level of binding of the reagent to the sample. In various alternatives, the binding reagent is a nucleic acid probe for a gene encoding the receptor or fragment thereof, an antibody which recognizes a receptor for IL-10 or a fragment thereof; or a ligand, agonist, or antagonist for a receptor for IL-10. Preferably the receptor is from a mammal, including a mouse or human.

The invention also provides methods of screening for a compound having binding affinity to a receptor for IL-10, comprising producing an isolated or recombinant receptor by a method as described; and assaying for the binding of the compound to the receptor, thereby identifying compounds having defined binding affinity therefor. Preferably, the compound is a ligand, agonist, or antagonist for these receptors.

The present invention also provides proteins and polypeptides, e.g., free of proteins with which they are naturally associated and having an amino acid sequence homologous to a fragment of a receptor for IL-10. Typically, the receptor is from a mammal, including a mouse or human, and specific embodiments have sequence of SEQ ID NO: 2 or 4.

The invention encompasses a recombinant or substantially pure polypeptides comprising a region exhibiting substantial identity to an amino acid sequence of a receptor for IL-10. Particular

embodiments include polypeptides having a sequence selected from SEQ ID NO: 2 or 4, or polypeptides attached to a solid support.

The present invention provides various antibodies having binding affinity to a recombinant receptor for IL-10, or a fragment thereof. Preferred embodiments are raised against the receptor for IL-10, and can be either neutralizing or non-neutralizing antibodies, fused to a toxic moiety, or conjugated to a marker moiety, including a radionuclide. Binding fragments such as Fab and FV are also provided. Preferably, the antibody or fragment binds to a receptor from a mammal, including a mouse or human.

Additionally, the invention provides methods of treating a host having a medical condition characterized by inappropriate IL-10 response or exhibiting abnormal expression of a receptor for IL-10, comprising administering to the host a therapeutically effective amount of a composition comprising (a) an antibody which binds to a receptor for IL-10 or fragment thereof; (b) a ligand, agonist, or antagonist for a receptor for IL-10; or (c) a ligand binding receptor, or fragment thereof, for IL-10. In one embodiment, the antibody is a monoclonal antibody or an antigen-binding fragment thereof. In others, the agonist or antagonist is selected by a method of contacting a target compound with (a) isolated or recombinant receptor for IL-10, or (b) ligand binding fragment of the receptor; and identifying the target compound with isolated or recombinant receptor for IL-10, or ligand binding fragment of the receptor; and identifying the target compound based upon the effects of the contacting.

The invention also provides methods of evaluating binding affinity of a test compound to a receptor for IL-10, the method comprising contacting (a) a sample containing the receptor, or a fragment thereof, with a labeled compound having known affinity for the receptor; and (b) the test compound; and measuring the level of bound labeled compound, the amount being inversely proportional to the amount of test compound which bound to the receptor. Preferably, the receptor is from a mammal, including a mouse or human. An alternative embodiment is a method of modulating biological activity of a receptor for IL-10, comprising contacting the receptor with a composition selected from an antibody which binds

to the receptor; a ligand, agonist, or antagonist for a receptor for IL-10; and a ligand binding fragment from a receptor for IL-10.

The invention also provides useful reagents in kit form. For example, it provides a kit useful for (a) quantifying a receptor for IL-10; or (b) for determining the binding affinity of a test sample to a receptor for IL-10; the kit comprising a labeled compound having binding affinity for the receptor, and a means for measuring bound labeled compound.

Various embodiments include kits further comprising recombinant receptor, wherein the labeled compound is a ligand for the receptor, including IL-10; wherein the compound is an antibody; wherein the means for measuring is a solid phase for immobilizing the receptor; or wherein the solid phase contains a capture molecule. The invention also provides a kit for assaying, in a sample, antibody against a receptor for IL-10, comprising the receptor and an antibody detection means. In one embodiment the receptor is attached to a solid support. Kits containing DNA probes for use in assaying, e.g., human IL-10 mRNA, are also provided.

The invention also provides compounds known to modulate activity of a receptor for IL-10, selected by a method of: contacting the compound with isolated or recombinant receptor, or a fragment thereof, for IL-10; and evaluating the effect on biological activity by the contacting.

The invention also provides methods of modulating a biological effect of IL-10, comprising a step of interfering with biological mechanisms, e.g., signal transduction, of a class 2 cytokine receptor, e.g., an interferon receptor. It also provides methods of modulating a biological effect of a class 2 receptor, e.g., an interferon, comprising a step of interfering with biological mechanisms of an IL-10 receptor.

DESCRIPTION OF THE INVENTION

All references cited herein are hereby incorporated in their entirety by reference.

The present invention provides amino acid and DNA sequences for mammalian IL-10 receptor subunits, exemplified by human and

mouse IL-10 receptor subunits. These sequences were obtained by screening pools of cells containing cDNA expression library products for specific binding to IL-10. Receptor-ligand complexes thus produced were chemically crosslinked, and methods were applied to isolate nucleic acids encoding the binding proteins.

The recombinant nucleic acids and isolated or purified nucleic acids are substantially homologous to a sequence encoding an IL-10 receptor subunit, or a fragment thereof. Nucleic acids encoding fusion polypeptides are also contemplated, as are vectors, transformed host cells, and organisms comprising such nucleic acids. Recombinant and isolated or purified IL-10 receptor subunits or fragments thereof derived from such nucleic acids are also a part of this invention.

The present invention also provides antibodies specific for epitopes on the receptor subunits. These include antibodies which bind specifically to epitopes that are common to receptors for IL-10 from different species, or epitopes that are unique to receptors from one species.

Kits comprising these materials are included herein. The various nucleic acids, polypeptides and antibodies in the kits can be used for various diagnostic or therapeutic purposes.

The various materials can be used in methods for treating mammals, particularly those suffering from undesired receptor function, e.g., autoimmune diseases, inappropriate immune responses of the T helper 2 class, inappropriate function of class II MHC, suppressed monocyte or macrophage-related immune functions, septic or toxic shock responses, and intracellular pathogen-mediated diseases. These methods comprise administering effective amounts of the materials, or contacting biological samples with them.

The materials of the present invention can also be used to select and screen for agonists and antagonists specific for the receptors. For example, soluble forms of the receptor subunits lacking the cytoplasmic and/or transmembrane domain can be prepared and immobilized by standard methods on solid supports, and used to specifically bind ligands. Ligands can thus be identified which specifically bind to the extracellular binding sites, or to the

intracellular domain of the IL-10 receptor. Of particular utility are ligands affecting multiple receptor types of the class to which IL-10 receptors belong, i.e., Class 2 receptor types. Class 2 receptors are described more fully below.

Antibodies can also be prepared which specifically bind to the ligand recognition sites, or to other regions of the receptors. The receptor subunits or fragments thereof, or synthetic polypeptides having sequences corresponding to subsequences of the subunits, can be used as antigens in conventional methods to produce such antibodies.

The descriptions below are primarily directed to either a mouse or a human IL-10 receptor, but most properties, both structural or biological, will be shared between them and other mammalian counterparts, e.g., rat, pig, sheep, goat, etc. Therefore, analogous uses and materials derived from other species can be obtained following the methods disclosed herein. Such other species may include other warm-blooded species such as birds or primates.

Standard methods are used herein, e.g., as described in Maniatis *et al.*,1982, *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Wu *et al.*, (eds),1989, "Recombinant DNA Methodology" from *Methods in Enzymology*, Academic Press, NY; Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, (2d ed.), vols 1-3, CSH Press, NY; Ausubel et al., *Biology*, Greene Publishing Associates, Brooklyn, NY; or Ausubel et al., 1987 and Supplements, *Current Protocols in Molecular Biology*, Greene/Wiley, New York.

To obtain nucleic acids encoding IL-10 receptor subunits, complementary DNA (cDNA) libraries were constructed using messenger RNA (mRNA) isolated from cells that were responsive to IL-10. A B cell line designated BJAB was used to make a cDNA library of human origin, and mast and macrophage cell lines designated MC/9 and J774, respectively, were used to make cDNA of mouse origin.

Several modifications and unique techniques had to be utilized to overcome problems associated with isolating cDNA clones by expression cloning. In particular, it was necessary to identify an

appropriate cell line from which the cDNA library encoding the desired IL-10 binding activity could be prepared. It was also important to establish whether IL-10 could bind to clonally-isolated expression products, and to select a cell line for expression which had low background IL-10 binding activity.

The IL-10 used as a ligand (the words "IL-10" and "ligand" are used interchangeably below) was modified by addition of an N-terminal extension which provided a means to detect a ligand-receptor crosslinked complex. The extension used was a FLAG peptide which was specifically recognized by an antibody, although other extensions could have been used instead. See Hopp *et al.*, *Bio/Technology* 6:1204 (1988). It was not known whether the extension would interfere with ligand-receptor interaction, or whether any IL-10 binding protein interactions observed would be physiologically relevant.

Through the use of the extension, it was possible to detect cells expressing a receptor component, and to affinity immobilize cells possessing a crosslinked complex on their surface. Both of these methods were applied to enrich and verify the identity of the IL-10 receptor subunits.

After cDNAs for receptor subunits from mouse and human cells were prepared, they were sequenced.

The present invention encompasses the unglycosylated receptor subunits actually sequenced, allelic variants of the protein and various metabolic variants, e.g., post-translational modifications, produced by different cell types, including natural cells and host cells used in recombinant expression systems. Various glycosylation variants and post-translational modification variants can be produced by choosing appropriate source cells.

Complete human IL-10 receptor subunit (IL-10R) nucleotide sequence and the predicted amino acid sequence are defined by SEQ ID NOs: 1 and 2, respectively. The mouse nucleotide sequence and the amino acid sequence predicted therefrom are defined by SEQ ID NOs: 3 and 4, respectively. The initiation codon in SEQ ID NO: 3 begins at base 61.

The human sequence was derived from a clone designated SW8.1, which was deposited in a plasmid with the American Type Culture Collection, Rockville, MD (ATCC) on December 4, 1992, and assigned accession number ATCC 69146. A hydrophobic membrane spanning segment appears to correspond to amino acids 217-243 of the human receptor component. Thus, a soluble binding fragment would correspond to one extending from about residues 1-216, or shorter.

The mouse sequence was derived from a clone designated pMR29, which was deposited in a plasmid with the ATCC on December 4, 1992, and assigned accession number ATCC 69147.

As used herein, the term "IL-10 receptor subunit" encompasses a protein or peptide comprising an amino acid sequence defined by SEQ ID NO: 2 or 4, or encoded by a nucleic acid sequence defined by SEQ ID NO: 1 or 3. This term also encompasses fragments of such proteins or polypeptides which specifically bind IL-10. Such fragments can be made by proteolytic cleavage, chemical synthesis or recombinant methods.

Some of the IL-10 receptor subunits of this invention bind to IL-10 such as human or mouse IL-10 with high affinity, e.g., at least about 10 nM, usually better than about 3 nM, preferably better than about 1 nM, and more preferably at better than about 0.5 nM. It is expected that the binding affinity of a multiprotein complex to the ligand will be higher when additional protein components associate with the component disclosed herein, e.g., an α-like chain.

The present invention also encompasses proteins or peptides having substantial amino acid sequence homology with the amino acid sequences defined herein but excludes any protein or peptide exhibiting substantially the same or lesser amino acid sequence homology than do known G-CSF, GM-CSF, EPO, TNF. IFN-γ, IL-2, IL-3, IL-4, IL-5, IL-6, or IL-7 receptor subunit sequences.

Some of the peptides do not bind IL-10 but may instead bind as yet uncharacterized intracellular molecules involved in signal transduction or other interactions with the receptors. These peptides may have amino acid sequences corresponding to sequences of the receptor intracellular or transmembrane domains.

Other peptides which may not have known binding capabilities are also provided. Because they have amino acid sequences corresponding to parts of the receptor molecules, these peptides are useful, e.g., as antigens for the production of antibodies.

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. This changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: [glycine, alanine]; [valine, isoleucine, leucine]; [aspartic acid, glutamic acid]; [asparagine, glutamine]; [serine, threonine]; [lysine, arginine]; and [phenylalanine, tyrosine]. Homologous amino acid sequences are intended to include natural allelic and interspecies variations in each respective receptor sequence.

Typical homologous proteins or peptides will have from 25-100% homology (if gaps can be introduced), to 50-100% homology (if conservative substitutions are included) with the amino acid sequences defined herein. Homology measures will be at least about 50%, generally at least 56%, more generally at least 62%, often at least 67%, more often at least 72%, typically at least 77%, more typically at least 82%, usually at least 86%, more usually at least 90%, preferably at least 93%, and more preferably at least 96%, and in particularly preferred embodiments, at least 98% or more. Some homologous proteins or peptides, such as the various receptor subtypes, will share various biological activities with the components of a receptor for IL-10, e.g., the embodiments used to illustrate this invention.

As used herein, the term "biological activity" is defined as including, without limitation, ligand (e.g., IL-10-like protein) binding, cross-reactivity with antibodies raised against each respective receptor component, and ligand dependent signal transduction. A "ligand-related activity" refers either to ligand binding itself, or to biological activities which are mediated by ligand binding, including, e.g., second messenger modulation, Ca^{++} sequestration, phosphorylation, protein associations, etc.

The term "ligand" refers to molecules, usually members of the family of cytokine-like peptides, that bind to the receptor via the segments involved in peptide ligand binding. Also, a ligand is a molecule which serves either as a natural ligand to which the receptor, or an analog thereof, binds, or a molecule which is a functional analog of a natural ligand. The functional analog may be a ligand with structural modifications, or may be a wholly unrelated molecule which has a molecular shape which interacts with the appropriate ligand binding determinants. The ligands may serve as agonists or antagonists, see, e.g., Goodman *et al.*, (eds), *Goodman & Gilman's: The Pharmacological Bases of Therapeutics* (8th ed), 1990, Pergamon Press.

Particularly useful soluble fragments of the receptor subunits bind the IL-10 ligand. Because the complete receptor appears to contain extracellular domains with which the ligand should bind, a protein comprising the extracellular segments amino proximal to the transmembrane helix segment running from residues 217-243 would exhibit such binding activity. Fusions of the extracellular domain with other proteins, and shorter segments can be easily tested for ligand binding activity. Fragments consisting of the intracellular domain may also be useful.

The human and mouse IL-10 receptor subunits exhibit 70-75% homology at the DNA and protein sequence levels. On the basis of distinctive structural motifs, the IL-10 receptor subunits are members of the class 2 group of the cytokine receptor superfamily. See, e.g., Bazan, *Immunology Today 11*:350 (1990); and Bazan, *Proc. Nat'l Acad. Sci. USA 87*:6934 (1990).

The characteristic motifs of the class 1 receptors include an amino-terminal set of four conserved cystines and one tryptophan residue, and a carboxy-terminal (membrane-proximal) collection of spaced aromatic residues. The motifs characteristic of the class 2 receptors are a conserved tryptophan and the second cysteine pair in the amino-terminal half, a WSxWS box analog in the carboxy-terminal half, and a second conserved cysteine pair.

The other members of class 2 are the receptors for interferon-α (IFN-α), interferon-γ (IFN-γ), tissue factor, and for a second soluble viral IFN receptor homolog. The IL-10 receptor components described herein are particularly closely related to the interferon-γ receptor. These domain structure similarities suggest that the mechanisms of action of IL-10 on its receptor may be similar to those involved in the interaction of IFN-γ with its receptor, although whether that is true or not is not material to this invention. See, e.g., Levy, *et al.*, *New Biologist* 2:923 (1990); Sen *et al.*, *J. Biol. Chem.* 267:5017 (1992); and Uze, *et al.*, *Proc. Nat'l Acad. Sci.* USA 89:4774 (1992).

For example, the antagonistic effect of IL-10 on macrophage activation by IFN-γ may directly intervene in the signal cascade of IFN response. This may be effected by interaction of a component in the IFN signal pathway with a component in the IL-10 pathway. Sharing of components in the two pathways is a real possibility, including direct structural overlap of one or more components in active receptor complexes, e.g., shared β-like subunits.

Alternatively, the structural similarities of the IFN and IL-10 receptor components will predict that regions of receptor structure critical in one pathway and conserved in the other will have like importance. This predictability extends to both ligand molecular surface shapes and to intracellular features likely to interact with downstream signal pathway components. This suggests methods of modulating a biological effect of IL-10, comprising a step of interfering with signal transduction of an interferon receptor, including, e.g., agonists or antagonists of an IFN, or homologous IL-10 receptor variants to IFN receptor mutants. Neutralizing antibodies to conserved regions would thus be expected to have similar effects on other receptors in the family.

This invention contemplates use of the isolated nucleic acids, e.g., DNA, or fragments which encode the IL-10 receptor subunits. Furthermore, this invention covers the use of isolated or recombinant DNA, or fragments thereof, which encode proteins which are homologous to each respective species variant or receptor or which was isolated using cDNA encoding a receptor for IL-10 as a probe.

The isolated DNA can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others known in the art. Such nucleic acids are generally useful as probes, e.g., for genes of mRNA encoding IL-10 receptors or fragments thereof.

An "isolated" nucleic acid as defined herein is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other components which naturally accompany a native sequence, e.g., ribosomes, polymerases, and flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some embodiments, contain minor heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants.

Thus, for example, products made by transforming cells with any unnaturally occurring vector are encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. This is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a

desired combination of functions not found in the commonly available natural forms.

A nucleic acid "fragment" is defined herein to be a contiguous segment of at least about 17 nucleotides, generally at least 21 nucleotides, more generally at least 25 nucleotides, ordinarily at least 30 nucleotides, more ordinarily at least 35 nucleotides, often at least 42 nucleotides, more often at least 49 nucleotides, typically at least 58 nucleotides, more typically at least 75 nucleotides, usually at least 100 nucleotides, more usually at least 200 nucleotides, preferably at least 300 nucleotides, more preferably at least 500 nucleotides, and in particularly preferred embodiments will be at least 800 or more nucleotides.

A DNA which codes for an IL-10 receptor subunit or a fragment thereof can be used to identify genes, mRNA and cDNA species which code for related or homologous receptors, as well as nucleic acids which code for species variants of these receptor components. Preferred probes for such use encode regions of the receptors which are conserved between different species variants. Conserved regions can be identified by sequence comparisons.

This invention further covers recombinant DNA molecules and fragments having a DNA sequence identical to or highly homologous to the isolated DNAs set forth herein. In particular, the sequences will often be operably linked to DNA segments which control transcription, translation, and DNA replication. Genomic sequences containing introns are also made available, along with methodologies to isolate them.

Homologous nucleic acid sequences, when compared, exhibit significant similarity. The standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison or based upon hybridization conditions. The hybridization conditions are described in greater detail below, but are further limited by the homology to other known receptors for cytokines, e.g., the above described receptor components. Homology measures will be limited, in addition to any stated parameters, to exceed any such similarity to these receptors, e.g., GM-CSF, IL-3, IL-4, and IL-5 receptor components.

Substantial nucleic acid sequence homology means either that the segments or their complementary strands are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 50% of the nucleotides, generally at least 56%, more generally at least 59%, ordinarily at least 62%, more ordinarily at least 65%, often at least 68%, more often at least 71%, typically at least 74%, more typically at least 77%, usually at least 80%, more usually at least about 85%, preferably at least about 90%, more preferably at least about 95 to 98% or more, and in particular embodiments, as high as about 99% or more of the nucleotides.

Substantial homology also exists when the segments will hybridize under selective (stringent) hybridization conditions to a strand or its complement, typically using a sequence defined herein.

The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 40 nucleotides, preferably at least about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides.

Stringent conditions, in referring to homology in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters typically controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, more usually in excess of about 37° C, typically in excess of about 45° C, more typically in excess of about 55° C, preferably in excess of about 65° C, and more preferably in excess of about 70° C. Stringent salt conditions will ordinarily be less than about 1000 mM, often less than about 700 mM, usually less than about 500 mM, more usually less than about 400 mM, typically less than about 300 mM, preferably less than about 200 mM, and more preferably less than about 150 mM. However, the combination of parameters is much more important than the measure of any single parameter [Wetmur *et al., J. Mol. Biol. 31*:349 (1968)].

Nucleic acids isolated and characterized as described herein can be used to make variants and mutants. They also can be used to make vector constructs useful, e.g., for making transgenic cells, including homologous recombination, e.g., gene "knock-out" animals, and for gene therapy. See, e.g., Goodnow, "Transgenic Animals" in Roitt (ed.), *Encyclopedia of Immunology*, 1992, Academic Press, San Diego, pp. 1502-1504; Travis, *Science 256*:1392 (1992); Kuhn, *et al., Science 254*:707 (1991); Capecchi, *Science 244*:1288 (1989); Robertson, (ed.), *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, 1987, IRL Press, Oxford; and Rosenberg, *J. Clinical Oncology 10*:180 (1992).

The isolated receptor DNAs can be readily modified by nucleotide substitutions, deletions, insertions and inversions. Preferably, IL-10 binding capability is maintained in expression products. Mutant receptors thus produced can readily be tested for specific binding to IL-10 as disclosed herein. These modified sequences can be prepared using well known methods such as site-specific mutagenesis. Modified sequences can also be prepared, e.g., using modified primers, the sequences described herein, and the polymerase chain reaction (PCR).

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from the receptor subunits. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product of an immunoglobulin with a receptor polypeptide is a continuous protein molecule having sequences fused in a typical peptide linkage, e.g., typically made as a single translation product and exhibiting properties derived from each source peptide. A similar concept applies to heterologous nucleic acid sequences.

In addition, new constructs may be made from combining similar functional domains from other proteins. For example, ligand-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham *et al., Science 243*:1330 (1989); and O'Dowd, *et al., J. Biol. Chem. 263*:15985 (1988). Thus, new chimeric polypeptides

exhibiting new combinations of specificities will result from the functional linkage of ligand-binding specificities and intracellular regions. For example, the ligand binding domains from other related receptors may be added or substituted for other binding domains of these receptors. The resulting protein will often have hybrid function and properties.

The phosphoramidite method described by Beaucage *et al.*, *Tetra. Letts.* 22:1859 (1981) will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

The present invention provides means to produce fusion proteins. Various receptor variants may have slightly different functions or biological activities, even though they share significant structural similarities. Dissection of structural elements which effect the various physiological functions or biological activities provided by the receptors is possible using standard techniques of modern molecular biology, particularly in comparing variants within the related family of cytokine receptors. See, e.g., the homolog-scanning mutagenesis technique described in Cunningham *et al.*, *supra*, and approaches used in O'Dowd *et al.*, *supra*, and Lechleiter *et al.*, *EMBO J.* 9:4381 (1990).

Ligand binding segments can be substituted between receptors to determine what structural features are important in both ligand binding affinity and specificity. The segments of receptor accessible to an extracellular ligand would be primary targets of such analysis. An array of different receptor variants, e.g., allelic, will be used to screen for ligands exhibiting combined properties of interaction with them. Intracellular functions would probably involve segments of the receptor which are normally accessible to the cytosol. However, receptor internalization may occur under certain circumstances, and interaction between intracellular components and the designated "extracellular" segments may occur. These intracellular functions usually involve signal transduction from ligand binding. The specific

segments of interaction of receptor with other proteins may be identified by mutagenesis or direct biochemical means, e.g., cross-linking or affinity methods. Structural analysis by crystallographic or other physical methods will also be applicable. Identification of the similarities and differences between receptor variants exhibiting distinct functions will lead to new diagnostic and therapeutic reagents or treatments.

Nucleic acids which encode IL-10 receptor subunits or fragments thereof are available in the pMR29 and pSW8.1 clones, or can be obtained by chemical synthesis or by screening cDNA or genomic libraries prepared from cell lines or tissue samples.

Such nucleic acids can be expressed in a wide variety of host cells for the synthesis of a full-length receptor subunit or fragments of a receptor which can in turn, for example, be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified receptor molecules; and for structure/function studies. Each receptor or its fragments can be expressed in host cells that are transformed or transfected with appropriate expression vectors. These molecules can be substantially free of protein or cellular contaminants, other than those derived from the recombinant host, and therefore are particularly useful in pharmaceutical compositions when combined with a pharmaceutically acceptable carrier and/or diluent. The receptor, or portions thereof, may be expressed as fusions with other proteins.

Expression vectors are typically self-replicating DNA or RNA constructs containing, e.g., the desired receptor gene or its fragments, usually operably linked to suitable genetic control elements that are recognized in a suitable host cell. These control elements are capable of effecting expression within a suitable host. The specific type of control elements necessary to effect expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and

sequences that terminate transcription and translation. Expression vectors also usually contain an origin of replication that allows the vector to replicate independently of the host cell.

The vectors of this invention contain DNA which encodes a receptor for an IL-10-like peptide, or a fragment thereof encoding a biologically active receptor polypeptide. The DNA can be under the control of a viral promoter and can encode a selection marker. This invention further contemplates use of such expression vectors which are capable of expressing eukaryotic cDNA coding for a receptor in a prokaryotic or eukaryotic host, where the vector is compatible with the host and where the eukaryotic cDNA coding for the receptor is inserted into the vector such that growth of the host containing the vector expresses the cDNA in question. Usually, expression vectors are designed for stable replication in their host cells or for amplification to greatly increase the total number of copies of the desirable gene per cell. It is not always necessary to require that an expression vector replicate in a host cell, e.g., it is possible to effect transient expression of the IL-10 receptor or its fragments in various hosts using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of IL-10 receptor or its fragments into the host DNA by recombination.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. Expression vectors are specialized vectors which contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector, but other forms of vectors which serve an equivalent function and which are, or become, known in the art are suitable for use herein. See, e.g., Pouwels *et al.*, *Cloning Vectors: A Laboratory Manual*, 1985 and Supplements, Elsevier, N.Y.; and Rodriquez *et al.* (eds), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, 1988, Buttersworth, Boston.

Transformed cells are cells, preferably mammalian, that have been transformed or transfected with receptor vectors constructed using recombinant DNA techniques. Transformed host cells usually express the receptor or its fragments, but for purposes of cloning, amplifying, and manipulating its DNA, do not need to express the receptor. This invention further contemplates culturing transformed cells in a nutrient medium, thus permitting the receptor or fragments, e.g., a soluble protein, to accumulate in the culture. The receptor proteins can be recovered from the cells or from the culture medium.

For purposes of this invention, DNA sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression.

Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., *E. coli* and *B. subtilis*. Lower eukaryotes include yeasts, e.g., *S. cerevisiae* and *Pichia*, and species of the genus *Dictyostelium*. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

Prokaryotic host-vector systems include a wide variety of vectors for many different species. As used herein, *E. coli* and its vectors will be used generically to include equivalent vectors used in other prokaryotes. A representative vector for amplifying DNA is pBR322 or many of its derivatives. Vectors that can be used to express the receptor or its fragments include, but are not limited to,

such vectors as those containing the lac promoter (pUC-series); trp promoter (pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters (pOTS); or hybrid promoters such as ptac (pDR540). See Brosius et al., "Expression Vectors Employing Lambda-, trp-, lac-, and Ipp-derived Promoters", in Vectors: A Survey of Molecular Cloning Vectors and Their Uses, (eds. Rodriguez and Denhardt), 1988, Buttersworth, Boston, Chapter 10, pp. 205-236.

Lower eukaryotes, e.g., yeasts and *Dictyostelium*, may be transformed with IL-10 receptor sequence containing vectors. For purposes of this invention, the most common lower eukaryotic host is the baker's yeast, *Saccharomyces cerevisiae*. It will be used to generically represent lower eukaryotes although a number of other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the integrating type), a selection gene, a promoter, DNA encoding the receptor or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as the alcohol dehydrogenase 2 promoter or metallothionine promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YEp-series); integrating types (such as the YIp-series), or mini-chromosomes (such as the YCp-series).

Higher eukaryotic cells grown in tissue culture are often the preferred host cells for expression of the functionally active IL-10 receptor protein. In principle, any higher eukaryotic tissue culture cell line is workable, e.g., insect baculovirus expression systems, whether from an invertebrate or vertebrate source. However, mammalian cells are often preferred. Transformation or transfection and propagation of such cells has become a routine procedure. Examples of useful cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK) cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines.

Expression vectors for such cell lines usually include an origin of replication, a promoter, a translation initiation site, RNA splice sites (if genomic DNA is used), a polyadenylation site, and a transcription termination site. These vectors also usually contain a selection gene or amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors include pCDNA1 (Invitrogen, San Diego, CA); pCD [Okayama *et al., Mol. Cell Biol. 5*:1136 (1985)]; pMC1neo Poly-A [Thomas *et al., Cell 51*:503 (1987)]; and a baculovirus vector such as pAC 373 or pAC 610 [O'Reilly *et al., Baculovirus Expression Vectors: A Laboratory Manual*, 1992, Freeman & Co., N.Y.

It will often be desired to express a receptor polypeptide in a system which provides a specific or defined glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression system. However, the pattern will be modifiable by exposing the polypeptide, e.g., an unglycosylated form, to appropriate glycosylating proteins introduced into a heterologous expression system. For example, the receptor gene may be co-transformed with one or more genes encoding mammalian or other glycosylating enzymes. Using this approach, certain mammalian glycosylation patterns will be achievable in prokaryote or other cells.

The nucleic acids of the invention will provide useful source materials possessing high levels of receptor proteins. Cells expressing these proteins can be sources for protein purification, of the natural receptor forms, or variants thereof. In addition, purification segments can be fused to appropriate portions of the receptor to assist in isolation and production. For example, the FLAG sequence, or a functional equivalent, can be fused to the protein via a protease-removable sequence, allowing the FLAG sequence to be recognized by an affinity reagent, and the purified protein subjected to protease digestion to remove the extension.

Many other equivalent segments exist, e.g., poly-histidine segments possessing affinity for heavy metal column reagents. See, e.g., Hochuli, *Chemische Industrie 12*:69 (1989); Hochuli, "Purification of Recombinant Proteins with Metal Chelate Adsorbent" in Setlow (ed), *Genetic Engineering, Principle and Methods* 12:87, 1990, Plenum Press, N.Y.; and Crowe *et al.*, *QIAexpress: The High Level Expression & Protein Purification System*, 1992, QUIAGEN, Inc. Chatsworth, CA.

Moreover, appropriate host cells may be used to express the receptor proteins at high levels and under physiological conditions which may allow for desirable post-translational processing, e.g., glycosylation variants.

Having produced high level expression sources, standard protein purification techniques are applied to purify the IL-10 receptor components to near homogeneity. These will include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel *et al.*, 1987 and periodic supplements, *Current Protocols in Molecular Biology*; Deutscher, "Guide to Protein Purification" in *Methods in Enzymology* Vol 182, 1990, and other volumes in this series; and manufacturers' literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA.

This invention also provides salts and labeled derivatives of the IL-10 receptor subunits. Such derivatives may involve covalent or aggregative association with chemical moieties. These derivatives generally fall into three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, for example with cell membranes. Such covalent or aggregative derivatives are useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of IL-10 or other binding ligands.

For example, the IL-10 receptor or a soluble form thereof can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated Sepharose, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or

purification of anti-IL-10 receptor antibodies or IL-10. The IL-10 receptor can also be labeled with a detectable group, for example radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to another fluorescent moiety for use in diagnostic assays.

The solubilized IL-10 receptor of this invention can be used as an immunogen for the production of antisera or antibodies specific for the receptor or fragments thereof. The purified receptor can be used to screen monoclonal antibodies or antigen-binding fragments prepared by immunization with various forms of impure preparations containing the IL-10 receptor.

The term "antibodies" also encompasses antigen binding fragments of natural antibodies. The purified receptor can also be used as a reagent to detect any antibodies generated in response to the presence of elevated levels of IL-10 receptor or cell fragments containing the IL-10 receptor. Additionally, IL-10 receptor fragments may also serve as immunogens to produce the antibodies of the present invention, as described immediately below. For example, this invention contemplates antibodies having binding affinity to or being raised against the amino acid sequences defined herein, or fragments thereof.

In particular, this invention contemplates antibodies having binding affinity to or being raised against specific fragments which are predicted to lie outside of the lipid bilayer. These fragments should become readily apparent upon completion of the sequence of the human or mouse receptors. In addition, this invention covers fragments of the IL-10 receptor which are predicted to reside on the extracellular side of the membrane. Analysis of protein structure to identify membrane associated regions is described, e.g., in von Heijne, *J. Mol. Biol. 225*:487 (1992); and Fasman *et al.*, *Trends in Biochemical Sciences 15*:89 (1990).

Antibodies can be raised to the various species variants of the receptor subunits and fragments thereof, both in their naturally occurring forms and in their recombinant forms. Additionally, antibodies can be raised to IL-10 receptors in either their active forms or in their inactive forms, the difference being that antibodies

to the active receptor are more likely to recognize epitopes which are only present in the active receptor. Anti-idiotypic antibodies can also be prepared by standard methods.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of the IL-10 receptor can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective IL-10 receptors, or screened for agonistic or antagonistic IL-10 receptor activity.

These monoclonal antibodies will normally bind with at least a Kd of about 1 mM, more normally at least 300 μM, generally at least 100 μM, more generally at least 30 μM, ordinarily at least 10 μM, more ordinarily at least 3 μM, often at least 1 μM, more often at least 300 nM, typically at least 100 nM, more typically at least 30 nM, usually at least 10 nM, more usually at least 3 nM, preferably at least 1 nM, more preferably at least 300 pM, and in especially preferred embodiments at least 100 to 10 pM or better.

The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be potent antagonists that bind to the IL-10 receptor and inhibit ligand binding to the receptor or inhibit the ability of an IL-10-like peptide to elicit a biological response. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides so that when the antibody binds to the receptor, the cell itself is killed. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they can bind to the IL-10 receptor without inhibiting ligand binding. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying IL-10 or IL-10 receptors.

Receptor fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. The IL-10 receptor and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. For descriptions of methods of preparing polyclonal antisera, see *Microbiology*, Hoeber Medical Division, Harper and Row, 1969; Landsteiner, *Specificity of Serological Reactions*, 1962, Dover Publications, New York; and Williams *et al., Methods in Immunology and Immunochemistry*, Vol. 1, 1967, Academic Press, New York.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, cows, sheep, goats, donkeys, primates, humans, etc. Descriptions of techniques used for preparing such monoclonal antibodies may be found in, e.g., Stites *et al.* (eds), *Basic and Clinical Immunology*, 4th ed., Lange Medical Publications, Los Altos, CA; Harlow and Lane, *Antibodies: A Laboratory Manual*, 1988, CSH Press; Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed), 1986, Academic Press, New York; and particularly in Kohler and Milstein, *Nature 256*:495 (1975).

Briefly, this method involves injecting an animal with an immunogen. The animal is then sacrificed and cells taken from its spleen, which are then fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing *in vitro*. The population of hybridomas is then screened to isolate individual clones, each of which secretes a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

Other suitable techniques involve *in vitro* exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See Huse *et al.*, "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," *Science 246*:1275 (1989); and Ward *et al.*, *Nature 341*:544 (1989).

The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents disclosing the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced, see Cabilly, U.S. Patent No. 4,816,567; and Moore *et al.*, U.S. Patent No. 4,642,334.

The antibodies of this invention can be used for affinity chromatography in isolating the receptor. Columns can be prepared where the antibodies are linked to a solid support, e.g., particles, such as agarose, Sephadex, or the like, where a cell lysate may be passed through the column, the column washed, followed by increasing concentrations of a mild denaturant, whereby the purified receptor protein will be released.

The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety, allowing easy detection of presence of antigen by antibody binding. The anti-idiotypic antibodies are useful for detecting or diagnosing various immunological conditions related to expression of the respective receptors.

Soluble receptor fragments can also be used as carriers for IL-10, e.g., to protect the cytokine from various degradative or other activities. The complex may be useful in certain situations as a slow release composition, allowing slow functional release of the cytokine or antagonist. Moreover, as an antagonist of IL-10, soluble forms of the receptor, e.g., a fragment containing the cytokine binding portions without membrane associated segments, will be useful diagnostic or therapeutic compositions. As a diagnostic reagent, such

fragment may be used as a substitute for antibodies against IL-10, but will likely be equivalent to a neutralizing antibody.

In addition, it is likely that the isolated component described herein is analogous to α subunits of other cytokine receptors. This suggests that a unique β component for the IL-10 receptor may exist, and could, in association with these components, modulate the activity from IL-10 binding. This will provide a convenient means to isolate this putative β subunit. See, e.g., Hayashida *et al., Proc. Nat'l. Acad. Sci.* USA *87*:9655 (1990). Alternatively, species or tissue specific accessory molecules, e.g., proteins, may provide a context for modification of the receptor protein properties or activities.

Both the naturally occurring and the recombinant form of the IL-10 receptor subunits of this invention are useful in kits and assay methods which are capable of screening compounds for binding activity to the receptors. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds per year. See, e.g., Fodor *et al., Science 251*:767 (1991), which describes methods for testing of binding affinity by a plurality of defined polymers synthesized on a solid substrate. Phage or other libraries of various random polypeptide sequences would also be useful. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble receptor such as is provided by this invention.

For example, antagonists can normally be found once the receptor has been characterized. Testing of potential receptor antagonists is now possible upon the development of highly automated assay methods using a purified receptor. In particular, new agonists and antagonists will be discovered using screening techniques made available by the reagents provided herein.

This invention is particularly useful for screening compounds by using the recombinant receptors in any of a variety of drug screening techniques. The advantages of using a recombinant receptor in screening for receptor reactive drugs include:

(a) improved renewable source of the receptor from a specific source;

(b) potentially greater number of receptors per cell giving better

signal-to-noise ratio in assays; and (c) species variant specificity (theoretically giving greater biological and disease specificity).

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing the receptor. Cells may be isolated which express a receptor in isolation from any others. Such cells, either in viable or fixed form, can be used for standard receptor/ligand binding assays. See Parce *et al.*, *Science 246*:243 (1989); and Owicki *et al.*, *Proc. Nat'l. Acad. Sci.* USA *87*:4007 (1990).

Competitive assays are particularly useful, where the cells (a source of IL-10 receptor) are contacted and incubated with a labeled ligand having known binding affinity to the receptor, such as ^{125}I-IL-10, and a test compound whose binding affinity to the IL-10 receptor is being measured. The bound ligand and free ligand are then separated to assess the degree of ligand binding. The amount of test compound bound is inversely proportional to the amount of labeled ligand binding measured. Any one of numerous techniques can be used to separate bound from free ligand to assess the degree of ligand binding. This separation step could typically involve a procedure such as adhesion to filters followed by washing, adhesion to plastic followed by washing, or centrifugation of the cell membranes.

Viable cells can also be used to screen for the effects of drugs on IL-10 receptor mediated functions, e.g., second messenger levels, i.e., Ca^{++}; cell proliferation; inositol phosphate pool changes; levels of phosphorylation; nitrous oxide levels; and others. Some detection methods allow for elimination of a separation step, e.g., a proximity sensitive detection system. Calcium sensitive dyes will be useful for detecting Ca^{++} levels, with a fluorimeter or a fluorescence cell sorting apparatus. See Lowenstein *et al.*, *Cell 70*:705 (1992).

Another method utilizes membranes from transformed eukaryotic or prokaryotic host cells as the source of the IL-10 receptor. These cells are stably transformed with DNA vectors directing the expression of the IL-10 receptor. Essentially, the membranes would be prepared from the cells and used in an

appropriate receptor/ligand binding assay, e.g., the competitive assay set forth above.

Still another approach is to use solubilized, unpurified or solubilized, purified receptors from transformed eukaryotic or prokaryotic host cells. This allows for a "molecular" binding assay with the advantages of increased specificity, the ability to automate, and high drug test throughput.

Another technique for drug screening involves an approach which provides high throughput screening for compounds having suitable binding affinity to the IL-10 receptor and is described in detail in Geysen, European Patent Application 84/03564. First, large numbers of different small peptide test compounds are synthesized on a solid substrate, e.g., plastic pins or some other appropriate surface. Then all the pins are reacted with solubilized, unpurified or solubilized, purified IL-10 receptor, and washed. The next step involves detecting bound IL-10 receptor.

Rational drug design may also be based upon structural studies of the molecular shapes of the receptor and other effectors or ligands. Effectors may be other proteins which mediate other functions in response to ligand binding, or other proteins which normally interact with the receptor. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or NMR techniques (2 or 3 dimensional). These will provide guidance as to which amino acid residues form molecular contact regions.

Purified receptor can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to these receptors can be used as capture antibodies to immobilize the respective receptor on the solid phase.

The blocking of physiological responses to IL-10-like peptides may result from the inhibition of binding of the ligand to the receptor, likely through competitive inhibition. Thus, *in vitro* assays of the present invention will often use isolated membranes from cells expressing a recombinant receptor, soluble fragments comprising the ligand binding segments of these receptors, or fragments attached to solid phase substrates. These assays will also allow for the diagnostic

determination of the effects of either binding segment mutations and modifications, or ligand mutations and modifications, e.g., ligand analogues.

This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to the receptor or receptor fragments compete with a test compound for binding to the receptor. In this manner, the antibodies can be used to detect the presence of any polypeptide which shares one or more binding sites of the receptor and can also be used to occupy binding sites on the receptor that might otherwise be occupied by IL-10.

Additionally, neutralizing antibodies against the receptor and soluble fragments of the receptor which contain the ligand binding site can be used to inhibit IL-10 receptor function in, e.g., macrophages, B cells, T cells, or related cell types.

This invention also contemplates use of the IL-10 receptor, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for detecting the presence of the IL-10 receptor. Typically the kit will have a compartment containing either a defined receptor peptide or gene segment or a reagent which recognizes one or the other.

A kit for determining the binding affinity of a test compound to IL-10 receptor would typically comprise a test compound; a labeled compound, for example a ligand or antibody having known binding affinity for IL-10 receptor; a source of IL-10 receptor (naturally occurring or recombinant); and a means for separating bound from free labeled compound, such as a solid phase for immobilizing IL-10 receptor. Once compounds are screened, those having suitable binding affinity to the IL-10 receptor can be evaluated in suitable biological assays, as are well known in the art, to determine whether they act as agonists or antagonists. The availability of recombinant receptor polypeptides also provide well defined standards for calibrating such assays.

A preferred kit for determining the concentration of, for example, IL-10 receptor in a sample would typically comprise a labeled compound, e.g., ligand or antibody, having known binding affinity for the receptor, a source of IL-10 receptor (naturally

occurring or recombinant) and a means for separating the bound from free labeled compound, for example a solid phase for immobilizing the IL-10 receptor. Compartments containing reagents, and instructions, will normally be provided.

Antibodies, including antigen binding fragments, specific for the receptor or receptor fragments are useful in diagnostic applications to detect the presence of elevated levels of the receptor and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, and further can involve the detection of antigens related to the IL-10 receptor in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and receptor-ligand complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA) and the like. For example, unlabeled antibodies can be employed by using a second antibody which is labeled and which recognizes the antibody to the IL-10 receptor or to a particular fragment thereof. These assays have also been extensively discussed in the literature. See, e.g., Harlow *et al.*, *Antibodies: A Laboratory Manual*, 1988, CSH.

Anti-idiotypic antibodies may have similar use to diagnose presence of antibodies against a receptor, as such may be diagnostic of various abnormal states. For example, over- or inappropriate production of IL-10 receptor may result in various immunological reactions which may be diagnostic of abnormal receptor expression, particularly in proliferative cell conditions such as cancer.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody, or labeled receptor is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the contents after

use. Typically the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium having appropriate concentrations for performing the assay.

Any of the constituents of the drug screening and the diagnostic assays may be used without modification or may be modified in a variety of ways. For example, they may be labeled as described above.

There are also numerous methods of separating the bound from the free ligand, or alternatively the bound from the free test compound. The receptor can be immobilized on various matrices followed by washing. Suitable matrices include plastic such as an ELISA plate, filters, and beads. Methods of immobilizing the receptor to a matrix include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of receptor/ligand complex by any of several methods including those utilizing, e.g., an organic solvent such as polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described by Rattle *et al.* [*Clin. Chem. 30*:1457 (1984)], and double antibody magnetic particle separation described in U.S. Patent No. 4,659,678.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of a receptor for IL-10. These sequences can be used as probes for detecting abnormal levels of the receptor in defined cells of patients suspected of having, e.g., an autoimmune condition, inability to properly respond to infections or inflammation, or a proliferative cell condition like cancer. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature.

Normally an oligonucleotide probe should have at least about 14 nucleotides, usually at least about 18 nucleotides, and the polynucleotide probes may be up to several kilobases. Various labels

may be employed, most commonly radionuclides, particularly ^{32}P. However, other techniques may also be employed, such as using photoreactive or biotin modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like.

Alternatively, antibodies may be employed which can recognize specific duplexes, including DNA duplexes, RNA duplexes, DNA-RNA hybrid duplexes, or DNA-protein complexes. The antibodies in turn may be labeled and the assay carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected. The use of probes to the novel anti-sense RNA may be carried out in any conventional techniques such as nucleic acid hybridization, plus and minus screening, recombinational probing, hybrid released translation (HRT), and hybrid arrested translation (HART). This also includes amplification techniques such as polymerase chain reaction (PCR).

Diagnostic kits which also test for the qualitative or quantitative presence of other markers are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet *et al., Progress in Growth Factor Res. 1*:89 (1989).

This invention provides reagents with significant therapeutic value. The IL-10 receptor (naturally occurring or recombinant), fragments thereof and antibodies thereto, along with compounds identified as having binding affinity to the IL-10 receptor, should be useful in the treatment of various conditions, e.g., autoimmune conditions, septic and toxic shock conditions, and infectious conditions. See, e.g., Hsu *et al., Int'l Immunol. 4*:563 (1992); de Waal Malefyt *et al., J. Expt'l Med. 174*:1209 (1991); Fiorentino *et al., J. Immunol. 147*:3815 (1991); and Ishida *et al., J. Expt'l Med. 175*:1213 (1992). Additionally, this invention should have therapeutic value in any disease or disorder associated with abnormal expression or abnormal triggering of receptors for IL-10.

For example, it is believed that the IL-10 receptor likely plays a role in many basic regulatory processes in immune function. Agonists and antagonists of the cytokine will be developed using the present invention. See also, e.g., Harada *et al., J. Biol. Chem. 267*:22752 (1992), which identifies receptor segments which are useful in antagonizing receptor function.

Recombinant IL-10 receptor, including soluble fragments thereof, or IL-10 receptor antibodies can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, along with physiologically innocuous stabilizers and excipients. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, e.g., which are soluble, which are not complement-binding.

Drug screening using the IL-10 receptor or fragments thereof can be performed to identify compounds having binding affinity to the IL-10 receptor. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of IL-10. Likewise, a compound having intrinsic stimulating activity can activate the receptor and is thus an agonist in that it simulates the activity of IL-10. This invention further contemplates the therapeutic use of antibodies to the IL-10 receptor as antagonists.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman et al. (eds), *Goodman and Gilman's: The*

Pharmacological Bases of Therapeutics, 8th Ed., 1990, Pergamon Press; and *Remington's Pharmaceutical Sciences*, 17th ed., 1990, Mack Publishing Co., Easton, Penn.

Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the *Merck Index*, Merck & Co., Rahway, New Jersey. Because of the high affinity binding between IL-10 and its receptors, low dosages of these reagents would be initially expected to be effective. Thus, dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 100 fM (femtomolar), with an appropriate carrier. Slow release formulations, or slow release apparatus will often be utilized for continuous administration. The intracellular segments of the receptors, both the IL-10 receptor and related receptors will find additional uses as described in detail below.

The IL-10 receptor, fragments thereof, and antibodies to the receptor or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in any conventional dosage formulation. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical composition.

Such compositions comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier must be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Avis *et al.* (eds.), *Pharmaceutical Dosage Forms: Parenteral Medications*, 1993, Dekker, New York; Lieberman *et al.* (eds.), *Pharmaceutical Dosage Forms: Tablets*, 1990, Dekker, New York; and Lieberman *et al.* (eds.), *Pharmaceutical Dosage Forms: Disperse Systems*, 1990, Dekker, New York The therapeutic methods of this invention may be combined with or used in association with other chemotherapeutic or chemopreventive agents.

The materials of this invention can also be delivered by standard gene therapy techniques, including, e.g., direct DNA injection into tissues, the use of recombinant viral vectors and implantation of transfected cells. See, e.g., Rosenberg, *J. Clin. Oncol. 10*:180-199 (1992).

It is quite likely that additional subunits of the IL-10 receptor exist. IL-10 exhibits different specific activities (units per mg of protein) in different biological assays. For example, the specfic activity of IL-10 in cytokine synthesis inhibitory factor assays, where IL-10 acts on macrophages, is higher than that observed in costimulation of mouse thymocyte or mouse mast cell proliferation.

The human and mouse IL-10 receptors provided herein bind IL-10, although the ability of each component by itself to bind vIL-10 has not yet been demonstrated. The apparent Kd of the recombinant IL-10 receptor (100-400 pM) is considerably higher than the EC_{50} of IL-10 on macrophages and monocytes (5-20 pM). By analogy to related class 2 cytokine receptors, e.g., IFN-α, IFN-β, or IFN-γ, the structural motifs of which are similar, an accessory molecule might be required for signal transduction upon IL-10 binding.

Various approaches can be used to screen for such accessory components. These approaches include both physical affinity methods and activity screening. Similar affinity methods as used herein with human IL-10 can be used with vIL-10. Because vIL-10 is biologically active but has not been shown to bind to the subunits, some modified form of the receptor may exist. A FLAG-vIL-10

fusion construct should be useful in selective purification of cells containing such a receptor form.

One approach is to transfect libraries made from appropriate cells, e.g., cells capable of responding to vIL-10, to screen transfected cells which otherwise are non-responsive to v-IL-10; or fail to bind to vIL-10 (or the FLAG-vIL-10 fusion). Such a library of transfected cells could be screened using a FLAG-vIL-10 marker at a concentration too low to bind effectively to the receptor subunits of this invention. See, e.g., Kitamura *et al., Cell 66*:1165 (1991).

Alternatively, a FLAG-vIL-10 fusion construct can be used for panning or FACS separation, e.g., as described below. These techniques may be combined with cotransfection with the IL-10 component already isolated, e.g., to isolate accessory components which modify the binding properties. Components which increase ligand binding affinity upon association are particularly desired. cDNA clones isolated in this manner are characterized, e.g., by sequencing, and compared structurally to other subunits or accessory proteins identified in other receptors.

EXAMPLES

The present invention can be illustrated by the following, non-limiting Examples. Unless otherwise specified, percentages given below for solids in solid mixtures, liquids in liquids, and solids in liquids are on a wt/wt, vol/vol and wt/vol basis, respectively.

The following Examples show that high specific activity iodinated human IL-10 (hIL-10) can bind in a specific and saturable manner to IL-10 receptors in several mouse and human cell lines. MC/9 proliferation assays showed that this labeled protein retains greater than 50% biological activity. Molecular weight sizing of the purified, iodinated protein indicated that the protein exists predominantly as a dimer and in this form is capable of binding specifically to its receptor. A 37 kDa dimer of human IL-10, when examined by sodium dodecylsulfate polyacrylamide gel electrophoresis [SDS-PAGE: Laemmli, *Nature 227*:680 (1970)] under

reducing conditions, may be dissociated by detergents to a single 18 kDa species. This is consistent with the 37 kDa species representing a non-covalently linked dimer of the cytokine. Moreover, this suggests that active hIL-10 is a non-covalently linked dimer.

Screening for specific binding with several cell lines of mouse and human origin indicates that murine mast cell line MC/9 and human B lymphoma line JY have the highest number of accessible, e.g., unoccupied, receptors per cell. Human B cell lines Ramos and BH5, as well as erythroleukemia line TF-1, bind at a reduced level relative to MC/9 and JY, followed by human T cell and macrophage lines. These cell lines were chosen based on the reported observations that mast cells, macrophage/ monocytes, B cells, and T cells respond to IL-10. The TF-1 cell line, originally derived from an erythroleukemic patient, is dependent on IL-3, erythropoietin, or GM-CSF for long term growth. The cell line is also responsive to IL-4, IL-5 and IL-6 in proliferation assays. Despite the responsiveness of the TF-1 cell line to a variety of cytokines, no proliferative effects on TF-1 cells in response to hIL-10 either alone or in combination with other cytokines could be detected.

Kd values obtained from Scatchard analysis indicate that hIL-10 binds with relatively high affinity to its receptor on both mouse and human cells, and that there are between 100 and 300 unoccupied receptors per cell. Competition binding assays with human and murine IL-10 on the mouse mast cell line MC/9 and the human cell line JY demonstrated that while the mouse ligand is able to compete with binding of iodinated hIL-10 to the mouse cell line, it cannot do so with the human cell line. One explanation is that under the binding conditions employed, hIL-10 can recognize and bind to both the mouse and the human receptor, while the mouse IL-10 can only recognize the mouse receptor. Supporting this notion of species specificity of the mouse ligand in binding site-recognition is the absence of any significant biological cross-reactivity of murine IL-10 on human cells.

Example 1: General Methods

Cell Lines and Tissue Culture

MC/9 cells (ATCC# CRL1649) were routinely grown in Dulbecco's modified essential medium (DMEM) with 10% fetal bovine serum (FBS) containing 3-5% mitogen-stimulated spleen-conditioned media, 100 U/ml mIL-4, 10 U/ml Penicillin/ Streptomycin, 2 mM glutamine, 1 mM sodium pyruvate, 1 x MEM essential and non-essential amino acids, 1 x MEM vitamins, 50 μM β-mercaptoethanol, 6 mg/liter folic acid, 116 mg/liter L-arginine, and 36 mg/liter L-asparagine. TF-1 cells [Kitamura *et al., J. Cell. Physiol. 140*:323 (1989)] were grown in RPMI1640 with 10% FBS and 1 μg/liter mouse GM-CSF. JY cells (provided by J. de Vries, DNAX, Palo Alto, CA) were grown in DMEM with 10% FBS, 6 mM glutamine, and antibiotics. Other cell lines [Ramos (ATCC# CRL1596), WEHI 265.1 (TIB204), U937 (CRL1593), HL-60 (CCL240), JD (CRL8163), Jijoye (CCL87), THP-1 (TIB202), B-JAB (provided by J. Banchereau, Schering-Plough France), and BH-5 (provided by W. Tadmori, Schering-Plough Research Institute, SPRI)] were grown in RPMI with 10% FBS, 6 mM glutamine, and antibiotics. In addition, culture media for BH-5 and THP-1 cells were supplemented with 50 μM β-mercaptoethanol. All tissue culture reagents were from GIBCO (Gaithersburg, MD).

Fluorescence Activated Cell Sorting (FACS)

FACS was performed using standard methods on a Becton-Dickinson FACStar PLUS. See, e.g., Shapiro, *Practical Flow Cytometry* (2d ed.), 1988, Alan Liss, New York.

Cytokines and Antibodies.

Recombinant CHO-derived human IL-10 and IL-5, as well as *E. coli*-derived human GM-CSF, IFN-γ, and mouse IL-10 were supplied by Schering-Plough Research Institute (SPRI), New Jersey. The specific biological activity of these preparations were 2.3 x 10^7 units/mg for hIL-10 and 1.6 x 10^7 units/mg for mIL-10 as measured by the MC/9 proliferation assay (see below). Recombinant hIL-6 was purchased from Genzyme (Cambridge, MA). Monoclonal

antibodies to IL-10 and IL-5 were provided by J. Abrams [DNAX, Palo Alto, CA; see Abrams *et al., Immunol. Rev. 127*:5 (1992)] but could also be made by standard methods.

Iodination of Human IL-10

Purified hIL-10 protein was labeled using the Enzymobead radioiodination reagent (Bio-Rad, Richmond, CA), which is an immobilized preparation of lactoperoxidase and glucose oxidase, following the manufacturer's protocols. The purified protein was passed through a PD-10 column (Pharmacia LKB Biotechnology, Piscataway, NJ) to remove free label. Additional samples were also iodinated using the lactoperoxidase method (NEN Research Products, Boston, MA). Specific radioactivity obtained was in the range of 100-180 μCi/μg hIL-10. The iodinated material was then passed through a 120 ml Sephadex G-75 column (Pharmacia LKB) with 1.1 ml fractions collected in phosphate-buffered saline (PBS). TCA precipitation was performed by incubating aliquots of the fractions in 10% trichloroacetic acid for 1 hour at 4° C. Pellets formed after centrifugation were then counted in Clinigamma counter (Pharmacia LKB).

MC/9 Proliferation Assay

Biological activity of hIL-10 was determined using a colorimetric MTT dye-reduction assay. See, e.g., Tada, *et al., J. Immun. Meth. 93*:157 (1986); and Mosmann, *J. Immun. Meth. 65*:55 (1983). Briefly, 5 x 10^3 MC/9 cells per well in 100 μl of medium containing 100 U mIL-4/ml in a 96 microtiter well were treated for 48 hours with varying amounts of human IL-10. The hIL-10 standard was used at a maximum of 200 units/100 μl and two-fold serially diluted. Twenty-five microliters of 5 mg/ml MTT was added and incubated for 3 to 5 hours. The cells were then detergent-lysed in 10% SDS with 10 mM HCl and the plates were read for absorbance at 570 nm.

Binding Assays and Scatchard Analysis

Approximately 5 x 10^6 cells of each cell line tested were pelleted by centrifugation at 200 x g for 10 min., washed in PBS, and resuspended in 200 µl of binding buffer (PBS, 10% fetal calf serum, 0.1% NaN_3) containing 100-500 pM iodinated hIL-10. After incubation at 4° C for two hours in a rotary mixer, the cells were centrifuged at 200 x g for 10 minutes, resuspended in 100 µl of binding buffer without labeled hIL-10, layered over 200 µl of a 1:1 mixture of dibutyl- and dioctyl-phthalate oils in elongated microcentrifuge tubes, centrifuged at 400 x g for 5 minutes at 4°C, and quick-frozen in liquid nitrogen. The cell pellets were then cut and counted in a Clinigamma 1272 counter (Pharmacia LKB). Non-specific binding was determined by performing the binding in the presence of 500 to 1000-fold molar excess unlabeled hIL-10.

For saturation binding experiments, two-fold serial dilutions of approximately 600 pM solution of iodinated hIL-10 were used, with a parallel series done to determine non-specific binding. Scatchard analysis was performed on the data points obtained using the EBDA Program (Elsevier-Biosoft, Cambridge, U.K.). Antibody inhibition was performed under the above binding conditions, but with the addition of a 100-fold molar excess of each of the monoclonal antibodies. Cytokine specificity was determined under similar conditions but with the addition of 500-fold molar excess of the cytokines indicated.

Chemical Cross-linking

About 2 x 10^8 cells were incubated for 4 hours at 4°C in 2 ml of binding medium consisting of PBS, 0.1% NaN_3, 10% bovine serum albumin, and 200 pM ^{125}I-hIL-10 with or without 200 nM unlabeled hIL-10. The cells were washed twice with PBS and then resuspended in 1 ml of PBS. Ten microliters of 15 M stock 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; Pierce Chemical Co.) were added to the cell suspension, and the cells were incubated for 1 hour at room temperature with constant rocking.

The reaction was stopped by washing the cells twice with cold PBS and then adding 150 mM glycine, pH 7.2, buffered with Tris-HCl. The cells were collected by centrifugation and lysed by adding 1 ml of lysis buffer containing 10 mM Tris-HCl (pH 7.5), 140 mM NaCl, 2 mM EDTA, 1 mg/ml leupepton (Sigma), 2 mM Prefabloc SC (Boehringer Mannheim), 2 mM iodoacetamide (Sigma), 2 mM o-phenanthroline (Sigma) and 1% Triton X-100 (Sigma). The lysates were centrifuged at 10,000 x g for 20 minutes at 4°C.

The supernatants were harvested and incubated overnight at 4°C with rabbit anti-hIL-10 polyclonal antiserum which had been prepared by standard methods and presorbed to protein G resin (Pierce Chemical Co.). Each sample contained 20 µl of the resin. After the incubation, the resin was washed three times with PBS and resuspended in 20 µl of of SDS-PAGE buffer without reducing agent. Twenty microliters of each sample were then subjected to SDS-PAGE in a 4-15% gradient gel (Daiichi Chemical Co., Tokyo) under nonreducing conditions. A set of prestained molecular weight markers (Life Technologies, Inc.) was run in parallel to determine the size of the cross-linked complexes. After electrophoresis, the gel was dried and exposed to a Kodak XAR film for 48 hours at -80°C with two intensifying screens.

COS 7 Transfections

Five micrograms of plasmid DNA were mixed with 5 x 10^6 COS 7 cells in 250 µl of DMEM with 10% FBS and antibiotics in an electroporation cuvette (Bio-Rad, Richmond, CA). The cells were electroporated with a Bio-Rad Gene Pulser using 0.20 kV, with the capacitance set at 960 µF and the resistance at 200 ohms. After 10 minutes at room temperature, the cells were transferred to 10 cm dishes with 10 ml of complete medium and allowed to attach.

After an overnight incubation at 37° C, the medium was replaced with the same medium but without serum. Two days later, the cells were detached from the plates by incubating in PBS with 4 mM EDTA and 0.03% NaN_3, harvested, and used for binding assays. Approximately 1 x 10^6 cells were used for each binding determination.

Example 2: Preparation of Human and Mouse IL-10 Fusion Proteins with FLAG Sequences

Nucleic acid constructs encoding fusion proteins were prepared using standard molecular biology techniques. The FLAG sequence is recognized by commercially available antibodies (IBI-Kodak, Rochester, NY) and does not interfere significantly with the association of the IL-10 fusion protein with the binding protein, as measured in biological assays for IL-10 activity.

Example 3: Preparation of cDNA Libraries

cDNA libraries were constructed using standard techniques from cell lines which are sensitive to IL-10. See SuperScript Plasmid System for cDNA Systems and Plasmid Cloning, Life Technologies, BRL, Gaithersburg, MD. The BJAB B human cell line was used, as were the mouse MC/9 mast cell and J774 macrophage cell lines.

Example 4: Enrichment of Transformed Cells Expressing Elevated Amounts of IL-10 Binding Protein

Cells transfected with the cDNA libraries were subjected to FACS sorting using biotinylated fluorescent FLAG antibodies as markers. After exposing transformed cells to the antibodies, phycoerythrin-streptavidin (PE-streptavidin) was added. The marked cells were then analyzed by FACS to collect the 3-5% of cells expressing the greatest amount of IL-10 binding. Selected cells were used to make cDNA libraries, and the cells were subjected to three cycles of enrichment. It was thereby found that IL-10 can compete with the FLAG-IL-10 binding.

Cells which expressed IL-10 binding protein were selected by affinity purification, i.e., panning, on plates coated with anti-FLAG antibodies. Cells thus identified were subjected to multiple cycles of the panning procedure, and their exogenous vector inserts were isolated and characterized.

Example 5: Characterization of Nucleic Acid Encoding the IL-10 Binding Protein

The isolated inserts from both the human and mouse cDNA sources were further characterized by sequencing by standard methods.

Most of the cells after selection had a higher fluorescence intensity, and the binding signal was greatly diminished by competition with a 50-fold excess of IL-10.

Example 6: Biological Activity of Lactoperoxidase-labeled Human IL-10

Purified CHO-derived hIL-10 was iodinated to high specific activity (100 to 200 μCi/μg protein) using the lactoperoxidase method. Initial attempts to label CHO-derived hIL-10 with the IODO-GEN reagent (Pierce, Rockford, IL) resulted in protein of insufficient specific activity to be used in receptor characterization. The lactoperoxidase method yielded iodinated hIL-10 with a specific activity approximately five-fold higher than that obtained with IODO-GEN.

To determine whether the high specific activity labeled hIL-10 was biologically active, samples were examined for their ability to induce MC/9 cell proliferation by the method of Thompson-Snipes *et al.*, *J. Exp. Med. 173*:507 (1991). Using 50 ng/ml concentrations of each, the estimated activities for the labeled and unlabeled IL-10 were found to be 7.48×10^2 and 1.16×10^3 units/ml, respectively. The labeled IL-10 thus retained 64% of the biological activity. Assays of other samples of iodinated hIL-10 indicated routinely greater than 50% biological activity retention.

Example 7: Dimeric Character of The Active Form of Radiolabeled hIL-10

The labeled protein mixture, when passed through a Sephadex G-75 gel-filtration column, was resolved into three distinct species. This fractionation was found to be necessary to reduce background

binding to target cells. The largest species was a high molecular weight form which elutes with the excluded volume. The smallest species eluted between the lowest molecular weight standard (13.7 kDa) and the dye marker Bromophenol Blue.

Sizing with molecular weight standards showed the second species to be approximately 37 kDa, consistent with the predicted molecular weight for a hIL-10 dimer. SDS-PAGE of the three species revealed that the high molecular weight form ran as an aggregate between 43 kDa and 200 kDa. The second species migrated under these conditions at approximately 18 kDa, while the third species was not observed at all. The radioactivity associated with the largest and the second species was TCA precipitable while that associated with the small species was not.

Example 8: Binding of Radioiodinated Human IL-10 to Cellular Receptors.

Based on the observation that the radioiodinated hIL-10 was biologically active, fractionated samples were tested for their ability to bind specifically to candidate cell lines. MC/9 cells respond to hIL-10 by proliferation, so they were first used to determine the binding specificity of hIL-10. When the three species fractionated from the G-75 column were tested for binding to MC/9 cells, the 37 kDa species, but not the other two, was able to bind to a high degree; moreover, a 500-fold molar excess of unlabeled IL-10 protein could block greater than 90% of the labeled IL-10 binding.

To ascertain the specificity of hIL-10 binding to its receptor, other cytokines, as well as monoclonal antibodies to hIL-10, were tested for their ability to inhibit the binding of iodinated hIL-10 to its cell surface receptor. It was found that excess hIL-10 was capable of competing with labeled hIL-10 in binding to TF-1 cells. In contrast, hIL-5, hIL-4, IFN-γ, GM-CSF, and hIL-6 were ineffective in competition.

To further demonstrate that the binding of hIL-10 to TF-1 cells was specific, monoclonal antibodies to hIL-10 and hIL-5 were examined for their ability to block binding of iodinated hIL-10 to its receptor. Neutralizing monoclonal antibodies generated against

hIL-10 inhibited the binding of labeled hIL-10 to TF-1 cells, but an anti-human IL-5 monoclonal antibody did not.

Binding assays with a number of different cell lines indicated that hIL-10 was able to bind to most of these lines to varying extents. The highest degree of binding was seen with the mouse mast cell line MC/9 and the human B-lymphoma line JY. TF-1 (a human erythroleukemia line) as well as Ramos and BH5 (human B-lymphoma lines) showed a reduced level of binding relative to JY and MC/9. Human IL-10 bound to the other cell lines examined at relatively low levels. A binding assay with WEHI 265.1, a mouse monocytic cell line, also showed a relatively low level of binding.

Example 9: Affinity of Human IL-10 Binding to Cellular Receptors

To determine the binding affinity and estimate the number of binding sites/receptors per cell, typical saturation binding curves were carried out with JY and MC/9 cells. Maximal binding occurred at approximately 300 to 400 pM of labeled hIL-10 for both cell lines. Scatchard analyses of representative binding data provided linear graphs with slopes yielding a Kd of approximately 150 pM for the JY cell line and 49 pM for the MC/9 line. Bmax values obtained, which represented the maximal concentration of ligand bound to cells, were 4.0 pM and 7.5 pM for MC/9 and JY cells, respectively. Assuming that one hIL-10 dimer ligand molecule binds one receptor, it was estimated that there were approximately 100 unoccupied receptors per cell for MC/9 and 180 unoccupied receptors per cell for JY. From several independent experiments, the human IL-10 binding affinity for JY and MC/9 cells was approximately 50 to 170 pM, with between 100 and 300 unoccupied receptors per cell.

Example 10: Species Specificity of Human and Mouse IL-10 Receptor Binding

To examine the species-specificity of receptor binding, the ability of mouse and human IL-10 to compete with labeled human IL-10 for binding to mouse and human cell lines was examined. Because the specific biological activity of *E. coli*-derived murine

IL-10 was 60-70% of human IL-10, as determined by the MC/9 biological assay, the concentrations of human and murine IL-10 in the competition experiments were adjusted accordingly. Both mouse and human IL-10 were able to block the binding of labeled hIL-10 to the mouse MC/9 line. In contrast, human IL-10, but not mouse IL-10, was able to successfully compete with the binding of labeled hIL-10 to the human B lymphoma line JY.

Example 11: Multiple Complexes Produced after Chemical Cross-linking of Human IL-10 to Cellular Receptors

To estimate the size of hIL-10 receptor binding complexes, ^{125}I-hIL-10 was bound to JY and MC/9 (ATCC CRL1649) cells, and the cells were treated with EDC as described above. Because the number of hIL-10 receptors in both cell lines was low, the cell lysates were immunoprecipitated after cross-linking with anti-hIL-10 polyclonal antiserum to enrich the binding complexes.

Following SDS-PAGE and autoradiography carried out as described above, it was found that both the JY and MC/9 cells yielded hIL-10-specific binding complexes. A major form of binding complex having an estimated molecular weight of about 97 kDa was produced from both cell lines. The JY cells, but not the MC/9 cells, yielded two additional bands having estimated molecular weights of about 190 and 210 kDa.

A few minor bands were also seen which migrated between 68 and 43 kDa markers. These may have been degradation products of the larger complexes. Cross-linked ^{125}I-hIL-10 appeared as a band migrating between 43 and 29 kDa markers. Formation of all cross-linked complexes was completely inhibited in the presence of a 1000-fold molar excess of unlabeled hIL-10.

Example 12: Specificity of Binding to Human IL-10

COS7 cells were transfected with the human or mouse cDNA clones, allowed to express the vector for 72 hours, and tested for binding to radioiodinated human IL-10. Unlike the vector alone, the cloned receptor cDNA was able to confer specific binding ability for

human IL-10 on COS cells. Both the human and mouse clones were able to bind human IL-10.

Example 13: Preparation of Soluble and Fusion Derivatives of the Human IL-10 Receptor Subunit.

In the following Example, the SEQ ID NOs defining the various oligonucleotide primers used for PCR are disclosed. The nucleotide sequences of these primers can thus be found by referring to the Sequence Listing.

The fusion derivative used to illustrate this invention below is a protein containing the human IL-10 receptor extracellular domain, the human IL-4 intracellular domain, and either the human IL-10 or human IL-4 transmembrane domain. Such constructs are useful, e.g., for elucidation of the mechanism of signal transduction by the relevant cytokines.

To facilitate recombinant plasmid cloning in *E. coli* and high-yield expression in transfected COS cells, a derivative of the pSV.Sport vector (Life Technologies, Gaithersburg, MD) was first prepared. This was done by replacing a *Pst*I-*Cla*I (end filled) fragment containing the SV40 *ori* and early promoter from pSV.Sport with a *Pst*I-*Hin*dIII (end filled) fragment containing the SRα promoter and SV40 t antigen intron from plasmid pDSRG (ATCC 68233). The resulting plasmid was designated pSR.Sport.

Reconstruction of the Intracellular Domain (IC) of the Human IL-4 Receptor

Procedurally, the IC of hIL-4 was divided into two individual parts which were combined to form the IC. A *Bam*HI-*Pst*I fragment was synthesized by PCR using primers designated C3632CC (SEQ ID NO: 5) and C3633CC (SEQ ID NO: 6), and plasmid pME18S-hIL-4R (ATCC 68263) as the template. An original *Sau*3A site was thereby converted into a *Bam*HI site by a silent mutation, to facilitate cloning. This fragment was restricted by *Bam*HI and *Pst*I, cloned into pUC19 (GIBCO-BRL; Gaithersburg, MD), and verified by DNA sequencing.

Plasmid pME18S-hIL-4R was then treated with *Pst*I to release a 900 bp *Pst*I-*Pst*I fragment, which was inserted at the *Pst*I site of plasmid pUC19 that had been modified as described above. The resulting construct containing the complete human IL-4 IC was verified by DNA sequencing. The hIL-4 IC was thus reconstructed as a *Bam*HI------*Pst*I------*Pst*I insert in pUC19.

Reconstruction of the Extracellular Domain (EC) of the Human IL-10 Receptor

Construction of the EC was also accomplished by ligation of two restriction fragments. A *Kpn*I site was created by a silent mutation at base 346 (the third base of the codon for glycine 95) of the hIL-10 receptor subunit (SEQ ID NO: 1), to facilitate cloning. The 5' and 3' end fragments were individually synthesized by PCR and cloned into pUC19 as an *Eco*RI/*Sal*I--*Kpn*I fragment and a *Kpn*I--*Bst*EII-Stop/*Eco*RI/*Bam*HI fragment, respectively. Clone SW8.1 DNA (ATCC 69146) was used as the template for the synthesis of both fragments. Primers designated C3628CC (SEQ ID NO: 7) and C3629CC (SEQ ID NO: 8) were used to make the 5' fragment, while primers designated C3630CC (SEQ ID NO: 9) and C3631CC (SEQ ID NO: 10) were used to make the 3' fragment.

A *Bst*EII site was created by a silent mutation of base 757 (SEQ ID NO: 1) A stop codon was added at the end of the EC for construction of a soluble hIL-10 receptor that was to be cloned as an *Eco*RI-*Eco*RI fragment, as described below.

The *Eco*RI/*Sal*I--*Kpn*I and *Kpn*I--*Bst*EII-Stop/*Eco*RI/*Bam*HI fragments were verified by DNA sequencing, after which the *Kpn*I--*Bst*EII-Stop/*Eco*RI/*Bam*HI fragment was ligated to the other to form the EC of hIL-10R, an *Eco*RI/*Sal*I--*Kpn*I--*Bst*EII-Stop/*Eco*RI/*Bam*HI fragment.

Reconstruction of the Transmembrane Domain (TM)

DNA encoding the hIL-4R TM was synthesized as an *Eco*RI/*Bst*EII-*Bam*HI fragment by PCR using plasmid pME18S-hIL-4R (ATCC 68263: deposited March 20, 1990) as the template and primers designated C3634CC (SEQ ID NO: 11) and C3635CC (SEQ ID NO:

12). Silent changes were introduced to create the restriction sites, and the construct was verified by DNA sequencing after cloning the TM into pUC19.

Assembly of Full-length Chimeric Receptor DNA

The hIL-4R IC was excised from the vector described above as a *Bam*HI-*Hin*dIII fragment and inserted into the pUC19 vector already containing the hIL-10R EC. A synthetic TM from hIL-4R was then inserted into the pUC19 vector as a *Bst*EII-*Bam*HI fragment, to generate a plasmid containing full-length chimeric receptor DNA. This DNA was then excised from the plasmid, cloned as as *Sal*I------- *Hin*dIII fragment into expression vector pSR.Sport, and expressed to produce the chimeric receptor.

Soluble Human IL-10 Receptor

The *Eco*RI/*Sal*I--*Kpn*I--*Bst*EII-Stop/*Eco*RI/*Bam*HI fragment described above was excised from the vector as an *Eco*RI------*Eco*RI fragment and cloned into pDSRG for direct transfection, or excised as an *Eco*RI-end-filled and *Sal*I fragment and cloned into *Sal*I/*Sna*BI restricted pSR.Sport for co-transfection with pDSRG.

Purification and Characterization of Soluble Human IL-10 Receptor

A human IL-10 affinity column was constructed by cross-linking hIL-10 prepared by standard methods (See U.S. Patent No. 5,231,012 to Mosmann *et al.*) to N-hydroxysuccinimide ester-activated agarose gel beads. Conditioned medium from transfected cells producing the soluble receptor was applied to the column, and the column was washed with PBS containing 0.5 M NaCl and 0.1% octylglucoside. The column was then loaded with 2 M $MgCl_2$ (pH 7.5) to release bound soluble hIL-10 receptor, and the eluted product was analyzed by SDS-PAGE with silver staining.

A group of three bands, two major and one minor, was observed with apparent molecular weights of about 43 kDa. Western blot analysis with a polyclonal anti-receptor peptide (encompassing residues 147-168 of SEQ ID NO: 1) antiserum prepared by standard methods revealed three bands corresponding

to the bands detected by silver staining, indicating that all were hIL-10R-related. A control using pre-immune serum produced no detectable signal.

Since the hIL-10 receptor protein predicted amino acid sequence contains a number of potential N-glycosylation sites, the observed complexity of the purified receptor protein might have been due to variable glycosylation. To investigate this possibility, the eluted product was first dialyzed into PBS and then treated with endoglycanase F (N-glycanase) and analyzed, along with the untreated product, by electrophoresis and Western blotting.

In the glycanase-treated sample the three bands at about 43 kDa migrated together as a single band having an apparent molecular weight of about 25 kDa, which was the predicted size of the unglycosylated recombinant soluble hIL-10 receptor. In addition, amino-terminal sequencing of the elution product from the affinity column showed that the first fifteen amino acid residues corresponded to residues 22-36 (SEQ ID NO: 2) predicted from the nucleotide sequence of the hIL-10 receptor DNA. It therefore appears that the polypeptide backbone of the purified soluble receptor was homogeneous in terms of molecular size.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Schering Corporation
(B) STREET: One Giralda Farms
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(ii) TITLE OF INVENTION: Mammalian Receptors For Interleukin-10 (IL-10)

(iii) NUMBER OF SEQUENCES: 12

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Macintosh
(C) OPERATING SYSTEM: Macintosh 6.0.8
(D) SOFTWARE: Microsoft Word 5.1a

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/110,683
(B) FILING DATE: 23-AUG-1993

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/011,066
(B) FILING DATE: 29-JAN-1993

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/989,792
(B) FILING DATE: 10-DEC-1992

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3632 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AAAGAGCTGG AGGCGCGCAG GCCGGCTCCG CTCCGGCCCC GGACGATGCG GCGCGCCCAG   60

G ATG CTG CCG TGC CTC GTA GTG CTG CTG GCG GCG CTC CTC AGC CTC       106
  Met Leu Pro Cys Leu Val Val Leu Leu Ala Ala Leu Leu Ser Leu
    1               5                  10                  15

CGT CTT GGC TCA GAC GCT CAT GGG ACA GAG CTG CCC AGC CCT CCG TCT    154
Arg Leu Gly Ser Asp Ala His Gly Thr Glu Leu Pro Ser Pro Pro Ser
                20                  25                  30

GTG TGG TTT GAA GCA GAA TTT TTC CAC CAC ATC CTC CAC TGG ACA CCC    202
Val Trp Phe Glu Ala Glu Phe Phe His His Ile Leu His Trp Thr Pro
            35                  40                  45

ATC CCA AAT CAG TCT GAA AGT ACC TGC TAT GAA GTG GCG CTC CTG AGG    250
Ile Pro Asn Gln Ser Glu Ser Thr Cys Tyr Glu Val Ala Leu Leu Arg
        50                  55                  60

TAT GGA ATA GAG TCC TGG AAC TCC ATC TCC AAC TGT AGC CAG ACC CTG    298
Tyr Gly Ile Glu Ser Trp Asn Ser Ile Ser Asn Cys Ser Gln Thr Leu
    65                  70                  75

TCC TAT GAC CTT ACC GCA GTG ACC TTG GAC CTG TAC CAC AGC AAT GGC    346
Ser Tyr Asp Leu Thr Ala Val Thr Leu Asp Leu Tyr His Ser Asn Gly
 80                  85                  90                  95

TAC CGG GCC AGA GTG CGG GCT GTG GAC GGC AGC CGG CAC TCC AAC TGG    394
Tyr Arg Ala Arg Val Arg Ala Val Asp Gly Ser Arg His Ser Asn Trp
                100                 105                 110
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ACC GTC ACC AAC ACC CGC TTC TCT GTG GAT GAA GTG ACT CTG ACA GTT   442
Thr Val Thr Asn Thr Arg Phe Ser Val Asp Glu Val Thr Leu Thr Val
            115                 120                 125

GGC AGT GTG AAC CTA GAG ATC CAC AAT GGC TTC ATC CTC GGG AAG ATT   490
Gly Ser Val Asn Leu Glu Ile His Asn Gly Phe Ile Leu Gly Lys Ile
        130                 135                 140

CAG CTA CCC AGG CCC AAG ATG GCC CCC GCG AAT GAC ACA TAT GAA AGC   538
Gln Leu Pro Arg Pro Lys Met Ala Pro Ala Asn Asp Thr Tyr Glu Ser
    145                 150                 155

ATC TTC AGT CAC TTC CGA GAG TAT GAG ATT GCC ATT CGC AAG GTG CCG   586
Ile Phe Ser His Phe Arg Glu Tyr Glu Ile Ala Ile Arg Lys Val Pro
160                 165                 170                 175

GGA AAC TTC ACG TTC ACA CAC AAG AAA GTA AAA CAT GAA AAC TTC AGC   634
Gly Asn Phe Thr Phe Thr His Lys Lys Val Lys His Glu Asn Phe Ser
                180                 185                 190

CTC CTA ACC TCT GGA GAA GTG GGA GAG TTC TGT GTC CAG GTG AAA CCA   682
Leu Leu Thr Ser Gly Glu Val Gly Glu Phe Cys Val Gln Val Lys Pro
            195                 200                 205

TCT GTC GCT TCC CGA AGT AAC AAG GGG ATG TGG TCT AAA GAG GAG TGC   730
Ser Val Ala Ser Arg Ser Asn Lys Gly Met Trp Ser Lys Glu Glu Cys
        210                 215                 220

ATC TCC CTC ACC AGG CAG TAT TTC ACC GTG ACC AAC GTC ATC ATC TTC   778
Ile Ser Leu Thr Arg Gln Tyr Phe Thr Val Thr Asn Val Ile Ile Phe
    225                 230                 235

TTT GCC TTT GTC CTG CTG CTC TCC GGA GCC CTC GCC TAC TGC CTG GCC   826
Phe Ala Phe Val Leu Leu Leu Ser Gly Ala Leu Ala Tyr Cys Leu Ala
240                 245                 250                 255

CTC CAG CTG TAT GTG CGG CGC CGA AAG AAG CTA CCC AGT GTC CTG CTC   874
Leu Gln Leu Tyr Val Arg Arg Arg Lys Lys Leu Pro Ser Val Leu Leu
                260                 265                 270

TTC AAG AAG CCC AGC CCC TTC ATC TTC ATC AGC CAG CGT CCC TCC CCA   922
Phe Lys Lys Pro Ser Pro Phe Ile Phe Ile Ser Gln Arg Pro Ser Pro
            275                 280                 285

GAG ACC CAA GAC ACC ATC CAC CCG CTT GAT GAG GAG GCC TTT TTG AAG   970
Glu Thr Gln Asp Thr Ile His Pro Leu Asp Glu Glu Ala Phe Leu Lys
        290                 295                 300

GTG TCC CCA GAG CTG AAG AAC TTG GAC CTG CAC GGC AGC ACA GAC AGT  1018
Val Ser Pro Glu Leu Lys Asn Leu Asp Leu His Gly Ser Thr Asp Ser
    305                 310                 315

GGC TTT GGC AGC ACC AAG CCA TCC CTG CAG ACT GAA GAG CCC CAG TTC  1066
Gly Phe Gly Ser Thr Lys Pro Ser Leu Gln Thr Glu Glu Pro Gln Phe
320                 325                 330                 335
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CTC CTC CCT GAC CCT CAC CCC CAG GCT GAC AGA ACG CTG GGA AAC GGG  1114
Leu Leu Pro Asp Pro His Pro Gln Ala Asp Arg Thr Leu Gly Asn Gly
                340                 345                 350

GAG CCC CCT GTG CTG GGG GAC AGC TGC AGT AGT GGC AGC AGC AAT AGC  1162
Glu Pro Pro Val Leu Gly Asp Ser Cys Ser Ser Gly Ser Ser Asn Ser
            355                 360                 365

ACA GAC AGC GGG ATC TGC CTG CAG GAG CCC AGC CTG AGC CCC AGC ACA  1210
Thr Asp Ser Gly Ile Cys Leu Gln Glu Pro Ser Leu Ser Pro Ser Thr
        370                 375                 380

GGG CCC ACC TGG GAG CAA CAG GTG GGG AGC AAC AGC AGG GGC CAG GAT  1258
Gly Pro Thr Trp Glu Gln Gln Val Gly Ser Asn Ser Arg Gly Gln Asp
    385                 390                 395

GAC AGT GGC ATT GAC TTA GTT CAA AAC TCT GAG GGC CGG GCT GGG GAC  1306
Asp Ser Gly Ile Asp Leu Val Gln Asn Ser Glu Gly Arg Ala Gly Asp
400                 405                 410                 415

ACA CAG GGT GGC TCG GCC TTG GGC CAC CAC AGT CCC CCG GAG CCT GAG  1354
Thr Gln Gly Gly Ser Ala Leu Gly His His Ser Pro Pro Glu Pro Glu
                420                 425                 430

GTG CCT GGG GAA GAA GAC CCA GCT GCT GTG GCA TTC CAG GGT TAC CTG  1402
Val Pro Gly Glu Glu Asp Pro Ala Ala Val Ala Phe Gln Gly Tyr Leu
            435                 440                 445

AGG CAG ACC AGA TGT GCT GAA GAG AAG GCA ACC AAG ACA GGC TGC CTG  1450
Arg Gln Thr Arg Cys Ala Glu Glu Lys Ala Thr Lys Thr Gly Cys Leu
        450                 455                 460

GAG GAA GAA TCG CCC TTG ACA GAT GGC CTT GGC CCC AAA TTC GGG AGA  1498
Glu Glu Glu Ser Pro Leu Thr Asp Gly Leu Gly Pro Lys Phe Gly Arg
    465                 470                 475

TGC CTG GTT GAT GAG GCA GGC TTG CAT CCA CCA GCC CTG GCC AAG GGC  1546
Cys Leu Val Asp Glu Ala Gly Leu His Pro Pro Ala Leu Ala Lys Gly
480                 485                 490                 495

TAT TTG AAA CAG GAT CCT CTA GAA ATG ACT CTG GCT TCC TCA GGG GCC  1594
Tyr Leu Lys Gln Asp Pro Leu Glu Met Thr Leu Ala Ser Ser Gly Ala
                500                 505                 510

CCA ACG GGA CAG TGG AAC CAG CCC ACT GAG GAA TGG TCA CTC CTG GCC  1642
Pro Thr Gly Gln Trp Asn Gln Pro Thr Glu Glu Trp Ser Leu Leu Ala
            515                 520                 525

TTG AGC AGC TGC AGT GAC CTG GGA ATA TCT GAC TGG AGC TTT GCC CAT  1690
Leu Ser Ser Cys Ser Asp Leu Gly Ile Ser Asp Trp Ser Phe Ala His
        530                 535                 540

GAC CTT GCC CCT CTA GGC TGT GTG GCA GCC CCA GGT GGT CTC CTG GGC  1738
Asp Leu Ala Pro Leu Gly Cys Val Ala Ala Pro Gly Gly Leu Leu Gly
    545                 550                 555
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AGC TTT AAC TCA GAC CTG GTC ACC CTG CCC CTC ATC TCT AGC CTG CAG 1786
Ser Phe Asn Ser Asp Leu Val Thr Leu Pro Leu Ile Ser Ser Leu Gln
560 565 570 575

TCA AGT GAG TGACTCGGGC TGAGAGGCTG CTTTTGATTT TAGCCATGCC 1835
Ser Ser Glu

TGCTCCTCTG CCTGGACCAG GAGGAGGGCC CTGGGGCAGA AGTTAGGCAC GAGGCAGTCT 1895

GGGCACTTTT CTGCAAGTCC ACTGGGGCTG GCCCAGCCAG GCTGCAGGGC TGGTCAGGGT 1955

GTCTGGGGCA GGAGGAGGCC AACTCACTGA ACTAGTGCAG GGTATGTGGG TGGCACTGAC 2015

CTGTTCTGTT GACTGGGGCC CTGCAGACTC TGGCAGAGCT GAGAAGGGCA GGGACCTTCT 2075

CCCTCCTAGG AACTCTTTCC TGTATCATAA AGGATTATTT GCTCAGGGGA ACCATGGGGC 2135

TTTCTGGAGT TGTGGTGAGG CCACCAGGCT GAAGTCAGCT CAGACCCAGA CCTCCCTGCT 2195

TAGGCCACTC GAGCATCAGA GCTTCCAGCA GGAGGAAGGG CTGTAGGAAT GGAAGCTTCA 2255

GGGCCTTGCT GCTGGGGTCA TTTTTAGGGG AAAAAGGAGG ATATGATGGT CACATGGGGA 2315

ACCTCCCCTC ATCGGGCCTC TGGGGCAGGA AGCTTGTCAC TGGAAGATCT TAAGGTATAT 2375

ATTTTCTGGA CACTCAAACA CATCATAATG GATTCACTGA GGGGAGACAA AGGGAGCCGA 2435

GACCCTGGAT GGGGCTTCCA GCTCAGAACC CATCCCTCTG GTGGGTACCT CTGGCACCCA 2495

TCTGCAAATA TCTCCCTCTC TCCAACAAAT GGAGTAGCAT CCCCCTGGGG CACTTGCTGA 2555

GGCCAAGCCA CTCACATCCT CACTTTGCTG CCCCACCATC TTGCTGACAA CTTCCAGAGA 2615

AGCCATGGTT TTTTGTATTG GTCATAACTC AGCCCTTTGG GCGGCCTCTG GGCTTGGGCA 2675

CCAGCTCATG CCAGCCCCAG AGGGTCAGGG TTGGAGGCCT GTGCTTGTGT TTGCTGCTAA 2735

TGTCCAGCTA CAGACCCAGA GGATAAGCCA CTGGGCACTG GGCTGGGGTC CCTGCCTTGT 2795

TGGTGTTCAG CTGTGTGATT TTGGACTAGC CACTTGTCAG AGGGCCTCAA TCTCCCATCT 2855

GTGAAATAAG GACTCCACCT TTAGGGGACC CTCCATGTTT GCTGGGTATT AGCCAAGCTG 2915

GTCCTGGGAG AATGCAGATA CTGTCCGTGG ACTACCAAGC TGGCTTGTTT CTTATGCCAG 2975

AGGCTAACAG ATCCAATGGG AGTCCATGGT GTCATGCCAA GACAGTATCA GACACAGCCC 3035

CAGAAGGGGG CATTATGGGC CCTGCCTCCC CATAGGCCAT TTGGACTCTG CCTTCAAACA 3095

AAGGCAGTTC AGTCCACAGG CATGGAAGCT GTGAGGGGAC AGGCCTGTGC GTGCCATCCA 3155

GAGTCATCTC AGCCCTGCCT TTCTCTGGAG CATTCTGAAA ACAGATATTC TGGCCCAGGG 3215

AATCCAGCCA TGACCCCCAC CCCTCTGCCA AAGTACTCTT AGGTGCCAGT CTGGTAACTG 3275

AACTCCCTCT GGAGGCAGGC TTGAGGGAGG ATTCCTCAGG GTTCCCTTGA AAGCTTTATT 3335

TATTTATTTT GTTCATTTAT TTATTGGAGA GGCAGCATTG CACAGTGAAA GAATTCTGGA 3395

TATCTCAGGA GCCCCGAAAT TCTAGCTCTG ACTTTGCTGT TTCCAGTGGT ATGACCTTGG 3455

AGAAGTCACT TATCCTCTTG GAGCCTCAGT TTCCTCATCT GCAGAATAAT GACTGACTTG 3515

TCTAATTCAT AGGGATGTGA GGTTCTGCTG AGGAAATGGG TATGAATGTG CCTTGAACAC 3575

AAAGCTCTGT CAATAAGTGA TACATGTTTT TTATTCCAAT AAATTGTCAA GACCACA 3632

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 578 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Leu Pro Cys Leu Val Val Leu Leu Ala Ala Leu Leu Ser Leu Arg
1 5 10 15

Leu Gly Ser Asp Ala His Gly Thr Glu Leu Pro Ser Pro Pro Ser Val
20 25 30

Trp Phe Glu Ala Glu Phe Phe His His Ile Leu His Trp Thr Pro Ile
35 40 45

Pro Asn Gln Ser Glu Ser Thr Cys Tyr Glu Val Ala Leu Leu Arg Tyr
50 55 60

Gly Ile Glu Ser Trp Asn Ser Ile Ser Asn Cys Ser Gln Thr Leu Ser
65 70 75 80

Tyr Asp Leu Thr Ala Val Thr Leu Asp Leu Tyr His Ser Asn Gly Tyr
85 90 95

Arg Ala Arg Val Arg Ala Val Asp Gly Ser Arg His Ser Asn Trp Thr
100 105 110

Val Thr Asn Thr Arg Phe Ser Val Asp Glu Val Thr Leu Thr Val Gly
115 120 125

Ser Val Asn Leu Glu Ile His Asn Gly Phe Ile Leu Gly Lys Ile Gln
130 135 140

Leu Pro Arg Pro Lys Met Ala Pro Ala Asn Asp Thr Tyr Glu Ser Ile
145 150 155 160

Phe Ser His Phe Arg Glu Tyr Glu Ile Ala Ile Arg Lys Val Pro Gly
165 170 175

Asn Phe Thr Phe Thr His Lys Lys Val Lys His Glu Asn Phe Ser Leu
180 185 190

Leu Thr Ser Gly Glu Val Gly Glu Phe Cys Val Gln Val Lys Pro Ser
195 200 205

Val Ala Ser Arg Ser Asn Lys Gly Met Trp Ser Lys Glu Glu Cys Ile
210 215 220

Ser Leu Thr Arg Gln Tyr Phe Thr Val Thr Asn Val Ile Ile Phe Phe
225 230 235 240

Ala Phe Val Leu Leu Leu Ser Gly Ala Leu Ala Tyr Cys Leu Ala Leu
245 250 255

Gln Leu Tyr Val Arg Arg Arg Lys Lys Leu Pro Ser Val Leu Leu Phe
260 265 270

Lys Lys Pro Ser Pro Phe Ile Phe Ile Ser Gln Arg Pro Ser Pro Glu
275 280 285

Thr Gln Asp Thr Ile His Pro Leu Asp Glu Glu Ala Phe Leu Lys Val
290 295 300

Ser Pro Glu Leu Lys Asn Leu Asp Leu His Gly Ser Thr Asp Ser Gly
305 310 315 320

Phe Gly Ser Thr Lys Pro Ser Leu Gln Thr Glu Glu Pro Gln Phe Leu
325 330 335

Leu Pro Asp Pro His Pro Gln Ala Asp Arg Thr Leu Gly Asn Gly Glu
340 345 350

Pro Pro Val Leu Gly Asp Ser Cys Ser Ser Gly Ser Ser Asn Ser Thr
355 360 365

Asp Ser Gly Ile Cys Leu Gln Glu Pro Ser Leu Ser Pro Ser Thr Gly
370 375 380

Pro Thr Trp Glu Gln Gln Val Gly Ser Asn Ser Arg Gly Gln Asp Asp
385 390 395 400

Ser Gly Ile Asp Leu Val Gln Asn Ser Glu Gly Arg Ala Gly Asp Thr
405 410 415

Gln Gly Gly Ser Ala Leu Gly His His Ser Pro Pro Glu Pro Glu Val
420 425 430

Pro Gly Glu Glu Asp Pro Ala Ala Val Ala Phe Gln Gly Tyr Leu Arg
435 440 445

Gln Thr Arg Cys Ala Glu Glu Lys Ala Thr Lys Thr Gly Cys Leu Glu
450 455 460

Glu Glu Ser Pro Leu Thr Asp Gly Leu Gly Pro Lys Phe Gly Arg Cys
465 470 475 480

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Leu Val Asp Glu Ala Gly Leu His Pro Pro Ala Leu Ala Lys Gly Tyr
                485                 490                 495

Leu Lys Gln Asp Pro Leu Glu Met Thr Leu Ala Ser Ser Gly Ala Pro
            500                 505                 510

Thr Gly Gln Trp Asn Gln Pro Thr Glu Glu Trp Ser Leu Leu Ala Leu
        515                 520                 525

Ser Ser Cys Ser Asp Leu Gly Ile Ser Asp Trp Ser Phe Ala His Asp
    530                 535                 540

Leu Ala Pro Leu Gly Cys Val Ala Ala Pro Gly Gly Leu Leu Gly Ser
545                 550                 555                 560

Phe Asn Ser Asp Leu Val Thr Leu Pro Leu Ile Ser Ser Leu Gln Ser
                565                 570                 575

Ser Glu
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3520 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CCATTGTGCT GGAAAGCAGG ACGCGCCGGC CGGAGGCGTA AAGGTCGGCT CCAGTGGACG   60

ATGCCGCTGT GCGCCCAGG ATG TTG TCG CGT TTG CTC CCA TTC CTC GTC ACG   112
                     Met Leu Ser Arg Leu Leu Pro Phe Leu Val Thr
                       1               5                  10

ATC TCC AGC CTG AGC CTA GAA TTC ATT GCA TAC GGG ACA GAA CTG CCA   160
Ile Ser Ser Leu Ser Leu Glu Phe Ile Ala Tyr Gly Thr Glu Leu Pro
            15                  20                  25

AGC CCT TCC TAT GTG TGG TTT GAA GCC AGA TTT TTC CAG CAC ATC CTC   208
Ser Pro Ser Tyr Val Trp Phe Glu Ala Arg Phe Phe Gln His Ile Leu
        30                  35                  40

CAC TGG AAA CCT ATC CCA AAC CAG TCT GAG AGC ACC TAC TAT GAA GTG   256
His Trp Lys Pro Ile Pro Asn Gln Ser Glu Ser Thr Tyr Tyr Glu Val
    45                  50                  55

GCC CTC AAA CAG TAC GGA AAC TCA ACC TGG AAT GAC ATC CAT ATC TGT   304
Ala Leu Lys Gln Tyr Gly Asn Ser Thr Trp Asn Asp Ile His Ile Cys
60                  65                  70                  75
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AGA AAG GCT CAG GCA TTG TCC TGT GAT CTC ACA ACG TTC ACC CTG GAT   352
Arg Lys Ala Gln Ala Leu Ser Cys Asp Leu Thr Thr Phe Thr Leu Asp
                80                  85                  90

CTG TAT CAC CGA AGC TAT GGC TAC CGG GCC AGA GTC CGG GCA GTG GAC   400
Leu Tyr His Arg Ser Tyr Gly Tyr Arg Ala Arg Val Arg Ala Val Asp
            95                  100                 105

AAC AGT CAG TAC TCC AAC TGG ACC ACC ACT GAG ACT CGC TTC ACA GTG   448
Asn Ser Gln Tyr Ser Asn Trp Thr Thr Thr Glu Thr Arg Phe Thr Val
        110                 115                 120

GAT GAA GTG ATT CTG ACA GTG GAT AGC GTG ACT CTG AAA GCA ATG GAC   496
Asp Glu Val Ile Leu Thr Val Asp Ser Val Thr Leu Lys Ala Met Asp
    125                 130                 135

GGC ATC ATC TAT GGG ACA ATC CAT CCC CCC AGG CCC ACG ATA ACC CCT   544
Gly Ile Ile Tyr Gly Thr Ile His Pro Pro Arg Pro Thr Ile Thr Pro
140                 145                 150                 155

GCA GGG GAT GAG TAC GAA CAA GTC TTC AAG GAT CTC CGA GTT TAC AAG   592
Ala Gly Asp Glu Tyr Glu Gln Val Phe Lys Asp Leu Arg Val Tyr Lys
                160                 165                 170

ATT TCC ATC CGG AAG TTC TCA GAA CTA AAG AAT GCA ACC AAG AGA GTG   640
Ile Ser Ile Arg Lys Phe Ser Glu Leu Lys Asn Ala Thr Lys Arg Val
            175                 180                 185

AAA CAG GAA ACC TTC ACC CTC ACG GTC CCC ATA GGG GTG AGA AAG TTT   688
Lys Gln Glu Thr Phe Thr Leu Thr Val Pro Ile Gly Val Arg Lys Phe
        190                 195                 200

TGT GTC AAG GTG CTG CCC CGC TTG GAA TCC CGA ATT AAC AAG GCA GAG   736
Cys Val Lys Val Leu Pro Arg Leu Glu Ser Arg Ile Asn Lys Ala Glu
    205                 210                 215

TGG TCG GAG GAG CAG TGT TTA CTT ATC ACG ACG GAG CAG TAT TTC ACT   784
Trp Ser Glu Glu Gln Cys Leu Leu Ile Thr Thr Glu Gln Tyr Phe Thr
220                 225                 230                 235

GTG ACC AAC CTG AGC ATC TTA GTC ATA TCT ATG CTG CTA TTC TGT GGA   832
Val Thr Asn Leu Ser Ile Leu Val Ile Ser Met Leu Leu Phe Cys Gly
                240                 245                 250

ATC CTG GTC TGT CTG GTT CTC CAG TGG TAC ATC CGG CAC CCG GGG AAG   880
Ile Leu Val Cys Leu Val Leu Gln Trp Tyr Ile Arg His Pro Gly Lys
            255                 260                 265

TTG CCT ACA GTC CTG GTC TTC AAG AAG CCT CAC GAC TTC TTC CCA GCC   928
Leu Pro Thr Val Leu Val Phe Lys Lys Pro His Asp Phe Phe Pro Ala
        270                 275                 280

AAC CCT CTC TGC CCA GAA ACT CCC GAT GCC ATT CAC ATC GTG GAC CTG   976
Asn Pro Leu Cys Pro Glu Thr Pro Asp Ala Ile His Ile Val Asp Leu
    285                 290                 295
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GAG GTT TTC CCA AAG GTG TCA CTA GAG CTG AGA GAC TCA GTC CTG CAT  1024
Glu Val Phe Pro Lys Val Ser Leu Glu Leu Arg Asp Ser Val Leu His
300                 305                 310                 315

GGC AGC ACC GAC AGT GGC TTT GGC AGT GGT AAA CCA TCA CTT CAG ACT  1072
Gly Ser Thr Asp Ser Gly Phe Gly Ser Gly Lys Pro Ser Leu Gln Thr
                320                 325                 330

GAA GAG TCC CAA TTC CTC CTC CCT GGC TCC CAC CCC CAG ATA CAG GGG  1120
Glu Glu Ser Gln Phe Leu Leu Pro Gly Ser His Pro Gln Ile Gln Gly
            335                 340                 345

ACT CTG GGA AAA GAA GAG TCT CCA GGG CTA CAG GCC ACC TGT GGG GAC  1168
Thr Leu Gly Lys Glu Glu Ser Pro Gly Leu Gln Ala Thr Cys Gly Asp
        350                 355                 360

AAC ACG GAC AGT GGG ATC TGC CTG CAG GAG CCC GGC TTA CAC TCC AGC  1216
Asn Thr Asp Ser Gly Ile Cys Leu Gln Glu Pro Gly Leu His Ser Ser
    365                 370                 375

ATG GGG CCC GCC TGG AAG CAG CAG CTT GGA TAT ACC CAT CAG GAC CAG  1264
Met Gly Pro Ala Trp Lys Gln Gln Leu Gly Tyr Thr His Gln Asp Gln
380                 385                 390                 395

GAT GAC AGT GAC GTT AAC CTA GTC CAG AAC TCT CCA GGG CAG CCT AAG  1312
Asp Asp Ser Asp Val Asn Leu Val Gln Asn Ser Pro Gly Gln Pro Lys
                400                 405                 410

TAC ACA CAG GAT GCA TCT GCC TTG GGC CAT GTC TGT CTC CTA GAA CCT  1360
Tyr Thr Gln Asp Ala Ser Ala Leu Gly His Val Cys Leu Leu Glu Pro
            415                 420                 425

AAA GCC CCT GAG GAG AAA GAC CAA GTC ATG GTG ACA TTC CAG GGC TAC  1408
Lys Ala Pro Glu Glu Lys Asp Gln Val Met Val Thr Phe Gln Gly Tyr
        430                 435                 440

CAG AAA CAG ACC AGA TGG AAG GCA GAG GCA GCA GGC CCA GCA GAA TGC  1456
Gln Lys Gln Thr Arg Trp Lys Ala Glu Ala Ala Gly Pro Ala Glu Cys
    445                 450                 455

TTG GAC GAA GAG ATT CCC TTG ACA GAT GCC TTT GAT CCT GAA CTT GGG  1504
Leu Asp Glu Glu Ile Pro Leu Thr Asp Ala Phe Asp Pro Glu Leu Gly
460                 465                 470                 475

GTA CAC CTG CAG GAT GAT TTG GCT TGG CCT CCA CCA GCT CTG GCC GCA  1552
Val His Leu Gln Asp Asp Leu Ala Trp Pro Pro Pro Ala Leu Ala Ala
                480                 485                 490

GGT TAT TTG AAA CAG GAG TCT CAA GGG ATG GCT TCT GCT CCA CCA GGG  1600
Gly Tyr Leu Lys Gln Glu Ser Gln Gly Met Ala Ser Ala Pro Pro Gly
            495                 500                 505

ACA CCA AGT AGA CAG TGG AAT CAA CTG ACC GAA GAG TGG TCA CTC CTG  1648
Thr Pro Ser Arg Gln Trp Asn Gln Leu Thr Glu Glu Trp Ser Leu Leu
        510                 515                 520
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GGT GTG GTT AGC TGT GAA GAT CTA AGC ATA GAA AGT TGG AGG TTT GCC  1696
Gly Val Val Ser Cys Glu Asp Leu Ser Ile Glu Ser Trp Arg Phe Ala
    525                 530                 535

CAT AAA CTT GAC CCT CTG GAC TGT GGG GCA GCC CCT GGT GGC CTC CTG  1744
His Lys Leu Asp Pro Leu Asp Cys Gly Ala Ala Pro Gly Gly Leu Leu
540                 545                 550                 555

GAT AGC CTT GGC TCT AAC CTG GTC ACC CTG CCG TTG ATC TCC AGC CTG  1792
Asp Ser Leu Gly Ser Asn Leu Val Thr Leu Pro Leu Ile Ser Ser Leu
                560                 565                 570

CAG GTA GAA GAA TGACAGCGGC TAAGAGTTAT TTGTATTCCA GCCATGCCTG      1844
Gln Val Glu Glu
            575

CTCCCCTCCC TGTACCTGGG AGGCTCAGGA GTCAAAGAAA TATGTGGGTC CTTTTCTGCA 1904

GACCTACTGT GACCAGCTAG CCAGGCTCCA CGGGGCAAGG AAAGGCCATC TTGATACACG 1964

AGTGTCAGGT ACATGAGAGG TTGTGGCTAG TCTGCTGAGT GAGGGTCTGT AGATACCAGC 2024

AGAGCTGAGC AGGATTGACA GAGACCTCCT CATGCCTCAG GGCTGGCTCC TACACTGGAA 2084

GGACCTGTGT TTGGGTGTAA CCTCAGGGCT TTCTGGATGT GGTAAGACTG TAGGTCTGAA 2144

GTCAGCTGAG CCTGGATGTC TGCGGAGGTG TTGGAGTGGC TAGCCTGCTA CAGGATAAAG 2204

GGAAGGCTCA AGAGATAGAA GGGCAGAGCA TGAGCCAGGT TTAATTTTGT CCTGTAGAGA 2264

TGGTCCCCAG CCAGGATGGG TTACTTGTGG CTGGGAGATC TTGGGGTATA CACCACCCTG 2324

AATGATCAGC CAGTCAATTC AGAGCTGTGT GGCAAAAGGG ACTGAGACCC AGAATTTCTG 2384

TTCCTCTTGT GAGGTGTCTC TGCTACCCAT CTGCAGACAG ACATCTTCAT CTTTTTACTA 2444

TGGCTGTGTC CCCTGAATTA CCAGCAGTGG CCAAGCCATT ACTCCCTGCT GCTCACTGTT 2504

GTGACGTCAG ACCAGACCAG ACGCTGTCTG TCTGTGTTAG TACACTACCC TTTAGGTGGC 2564

CTTTGGGCTT GAGCACTGGC CCAGGCTTAG GACTTATGTC TGCTTTTGCT GCTAATCTCT 2624

AACTGCAGAC CCAGAGAACA GGGTGCTGGG CTGACACCTC CGTGTTCAGC TGTGTGACCT 2684

CCGACCAGCA GCTTCCTCAG GGGACTAAAA TAATGACTAG GTCATTCAGA AGTCCCTCAT 2744

GCTGAATGTT AACCAAGGTG CCCCTGGGGT GATAGTTTAG GTCCTGCAAC CTCTGGGTTG 2804

GAAGGAAGTG GACTACGGAA GCCATCTGTC CCCCTGGGGA GCTTCCACCT CATGCCAGTG 2864

TTTCAGAGAT CTTGTGGGAG CCTAGGGCCT TGTGCCAAGG GAGCTGCTAG TCCCTGGGGT 2924

CTAGGGCTGG TCCCTGCCTC CCTATACTGC GTTTGAGACC TGTCTTCAAA TGGAGGCAGT 2984

TTGCAGCCCC TAAGCAAGGA TGCTGAGAGA AGCAGCAAGG CTGCTGATCC CTGAGCCCAG 3044

AGTTTCTCTG AAGCTTTCCA AATACAGACT GTGTGACGGG GTGAGGCCAG CCATGAACTT 3104
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TGGCATCCTG CCGAGAAGGT CATGACCCTA ATCTGGTACG AGAGCTCCTT CTGGAACTGG 3164

GCAAGCTCTT TGAGACCCCC CTGGAACCTT TATTTATTTA TTTGCTCACT TATTTATTGA 3224

GGAAGCAGCG TGGCACAGGC GCAAGGCTCT GGGTCTCTCA GGAGGTCTAG ATTTGCCTGC 3284

CCTGTTTCTA GCTGTGTGAC CTTGGGCAAG TCACGTTTCC TCGTGGAGCC TCAGTTTTCC 3344

TGTCTGTATG CAAAGCTTGG AAATTGAAAT GTACCTGACG TGCTCCATCC CTAGGAGTGC 3404

TGAGTCCCAC TGAGAAAGCG GGCACAGACG CCTCAAATGG AACCACAAGT GGTGTGTGTT 3464

TTCATCCTAA TAAAAAGTCA GGTGTTTTGT GGAAAAAAAA AAAAAAAAAA AAAAAA 3520

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 575 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Leu Ser Arg Leu Leu Pro Phe Leu Val Thr Ile Ser Ser Leu Ser
1 5 10 15

Leu Glu Phe Ile Ala Tyr Gly Thr Glu Leu Pro Ser Pro Ser Tyr Val
20 25 30

Trp Phe Glu Ala Arg Phe Phe Gln His Ile Leu His Trp Lys Pro Ile
35 40 45

Pro Asn Gln Ser Glu Ser Thr Tyr Tyr Glu Val Ala Leu Lys Gln Tyr
50 55 60

Gly Asn Ser Thr Trp Asn Asp Ile His Ile Cys Arg Lys Ala Gln Ala
65 70 75 80

Leu Ser Cys Asp Leu Thr Thr Phe Thr Leu Asp Leu Tyr His Arg Ser
85 90 95

Tyr Gly Tyr Arg Ala Arg Val Arg Ala Val Asp Asn Ser Gln Tyr Ser
100 105 110

Asn Trp Thr Thr Thr Glu Thr Arg Phe Thr Val Asp Glu Val Ile Leu
115 120 125

Thr Val Asp Ser Val Thr Leu Lys Ala Met Asp Gly Ile Ile Tyr Gly
130 135 140

Thr Ile His Pro Pro Arg Pro Thr Ile Thr Pro Ala Gly Asp Glu Tyr
145 150 155 160

Glu Gln Val Phe Lys Asp Leu Arg Val Tyr Lys Ile Ser Ile Arg Lys
165 170 175

Phe Ser Glu Leu Lys Asn Ala Thr Lys Arg Val Lys Gln Glu Thr Phe
180 185 190

Thr Leu Thr Val Pro Ile Gly Val Arg Lys Phe Cys Val Lys Val Leu
195 200 205

Pro Arg Leu Glu Ser Arg Ile Asn Lys Ala Glu Trp Ser Glu Glu Gln
210 215 220

Cys Leu Leu Ile Thr Thr Glu Gln Tyr Phe Thr Val Thr Asn Leu Ser
225 230 235 240

Ile Leu Val Ile Ser Met Leu Leu Phe Cys Gly Ile Leu Val Cys Leu
245 250 255

Val Leu Gln Trp Tyr Ile Arg His Pro Gly Lys Leu Pro Thr Val Leu
260 265 270

Val Phe Lys Lys Pro His Asp Phe Phe Pro Ala Asn Pro Leu Cys Pro
275 280 285

Glu Thr Pro Asp Ala Ile His Ile Val Asp Leu Glu Val Phe Pro Lys
290 295 300

Val Ser Leu Glu Leu Arg Asp Ser Val Leu His Gly Ser Thr Asp Ser
305 310 315 320

Gly Phe Gly Ser Gly Lys Pro Ser Leu Gln Thr Glu Glu Ser Gln Phe
325 330 335

Leu Leu Pro Gly Ser His Pro Gln Ile Gln Gly Thr Leu Gly Lys Glu
340 345 350

Glu Ser Pro Gly Leu Gln Ala Thr Cys Gly Asp Asn Thr Asp Ser Gly
355 360 365

Ile Cys Leu Gln Glu Pro Gly Leu His Ser Ser Met Gly Pro Ala Trp
370 375 380

Lys Gln Gln Leu Gly Tyr Thr His Gln Asp Gln Asp Asp Ser Asp Val
385 390 395 400

Asn Leu Val Gln Asn Ser Pro Gly Gln Pro Lys Tyr Thr Gln Asp Ala
405 410 415

Ser Ala Leu Gly His Val Cys Leu Leu Glu Pro Lys Ala Pro Glu Glu
420 425 430

Lys Asp Gln Val Met Val Thr Phe Gln Gly Tyr Gln Lys Gln Thr Arg
435 440 445

Trp Lys Ala Glu Ala Ala Gly Pro Ala Glu Cys Leu Asp Glu Glu Ile
450 455 460

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Pro Leu Thr Asp Ala Phe Asp Pro Glu Leu Gly Val His Leu Gln Asp
465                 470                 475                 480

Asp Leu Ala Trp Pro Pro Pro Ala Leu Ala Ala Gly Tyr Leu Lys Gln
                485                 490                 495

Glu Ser Gln Gly Met Ala Ser Ala Pro Pro Gly Thr Pro Ser Arg Gln
            500                 505                 510

Trp Asn Gln Leu Thr Glu Glu Trp Ser Leu Leu Gly Val Val Ser Cys
        515                 520                 525

Glu Asp Leu Ser Ile Glu Ser Trp Arg Phe Ala His Lys Leu Asp Pro
    530                 535                 540

Leu Asp Cys Gly Ala Ala Pro Gly Gly Leu Leu Asp Ser Leu Gly Ser
545                 550                 555                 560

Asn Leu Val Thr Leu Pro Leu Ile Ser Ser Leu Gln Val Glu Glu
                565                 570                 575
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATGGTGGGAT CCGATTCCCA ACCCAGCCCG CAGCCGCCTC GT                    42
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
ACGCTCAGCA CTGCAGCTGC CCCATGCTGG AGGACAT                          37
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCAGCGAATT CGTCGACGCC GCCACCATGC TGCCGTGCCT CGTAGTGCT 49

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CACTCTGGCT CACCGGTACC CATTGCTGTG GTACAGGTCC AAGGTC 46

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTACCACAGC AATGGGTACC GGGCCAGAGT GCGGGCTGTG GAC 43

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 65 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCTTCAGTAG CTGGATCCGA ATTCTCAGTT GGTCACCGTG AAATACTGCC TGGTGAGGGA 60

GATGC 65

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAATTCGTGA GGTGACCAAC CTCCTGCTGG GCGTCAGCGT TTCCTGC 47

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCTCGACGAT GGATCCCACC ATTCTTTCTT AATCTTGGTG ATGC 44

WHAT IS CLAIMED IS:

1. An isolated nucleic acid which encodes a mammalian IL-10 receptor protein or a fragment thereof.

2. An isolated nucleic acid which hybridizes under stringent conditions to a plasmid deposited with the Americn Type Culture Collection under accession number ATCC 69146 or 69147.

3. A nucleic acid of either claim 1 or 2 which encodes a receptor protein or fragment that specifically binds IL-10.

4. A recombinant vector comprising a nucleic acid of any one of claims 1 to 3.

5. A nucleic acid or recombinant vector of any one of claims 1 to 4 in which the nucleic acid encodes a human or mouse IL-10 receptor protein or fragment.

6. A nucleic acid or recombinant vector of any one of claims 1 to 5 in which which the nucleic acid encodes a soluble form of a human IL-10 receptor protein.

7. A nucleic acid or recombinant vector of claim 5 in which the nucleic acid has a nucleotide sequence defined by SEQ ID NO: 1 or by SEQ ID NO: 3 or which, due to the degeneracy of the genetic code, is a functional equivalent of either of such sequences.

8. A host cell comprising a recombinant vector of any one of claims 4 to 7.

9. A method for producing a mammalian IL-10 receptor protein or a fragment thereof, comprising culturing a host cell of claim 8 under conditions in which the nucleic acid is expressed.

10. An isolated mammalian receptor protein or a fragment thereof which specifically binds IL-10.

11. A receptor protein or fragment of claim 10 which is a human or mouse IL-10 receptor protein or fragment.

12. A receptor protein of claim 11 which has an amino acid sequence defined by SEQ ID NO: 2 or SEQ ID NO: 4.

13. A fragment of either claim 10 or 11 which is a soluble form of a human IL-10 receptor protein.

14. A chimeric receptor protein comprising a human IL-10 receptor extracellular domain and a human IL-4 receptor intracellular domain.

15. An antibody or binding fragment thereof against a receptor protein or fragment of any one of claims 10 to 14.

16. An antibody or binding fragment of claim 15 which is a monoclonal antibody or binding fragment.

17. A pharmaceutical composition for antagonizing the biological activity of IL-10 comprising a pharmaceutically acceptable carrier and a soluble form of a human IL-10 receptor protein or an antibody or binding fragment of either claim 15 or 16.

18. A method for the manufacture of a pharmaceutical composition for antagonizing the biological activity of IL-10 comprising admixing a pharmaceutically acceptable carrier with a soluble form of a human IL-10 receptor protein or an antibody or binding fragment of either claim 15 or 16.

19. A kit comprising a container comprising a nucleic acid, receptor protein or fragment thereof, or antibody or binding fragment thereof of any one of claims 1 to 3, 10 to 13, 15 or 16.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 93/11638

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/12 C12N15/62 C07K13/00 C12P21/08 C12N5/10
A61K37/02 A61K39/395 C12Q1/68 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C07K A61K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 90, no. 23 , 1 December 1993 , WASHINGTON US pages 11267 - 11271 HO AS;LIU Y;KHAN TA;HSU DH;BAZAN JF;MOORE KW 'A receptor for interleukin 10 is related to interferon receptors' see the whole document --- -/--	1-19

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 1994	25 -03- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Nauche, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY. vol. 268, no. 28 , 5 October 1993 , BALTIMORE US pages 21053 - 21059 TAN JC;INDELICATO SR;NARULA SK;ZAVODNY PJ;CHOU CC 'Characterization of interleukin-10 receptors on human and mouse cells.' see the whole document	1-19
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 88 , February 1991 , WASHINGTON US pages 1172 - 1176 VIEIRA, P. ET AL.; 'Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones : Homology to Epstein-Barr virus open reading frame BCRFI'	